PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 November 2000 (06.11.00)	
International application No. PCT/US00/04745	**Applicant's or agent's file reference** 440200/PALL
International filing date (day/month/year) 25 February 2000 (25.02.00)	**Priority date** (day/month/year) 25 February 1999 (25.02.99)
Applicant HOU, Chung-Jen et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 September 2000 (07.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer S. Mafla
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
440200/PALL		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04745	25/02/2000	25/02/1999
International Patent Classification (IPC) or national classification and IPC B01D67/00		
Applicant PALL CORPORATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 13 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/09/2000	25.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Nissen, V Telephone No. +49 89 2399 8619

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/04745

I. Basis f the r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1,3-7,9-13,15,16, 18,20	as originally filed	
2,2a,8,8a,14,14a, 17,19	with telefax of	28/02/2001

Claims, No.:

41-47	as originally filed	
1-40	with telefax of	28/02/2001

Drawings, sheets:

1-3	as originally filed	

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21, 27-40
	No:	Claims	22-26
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-40

Industrial applicability (IA)	Yes:	Claims	1-40
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item VIII Certain observations on the international application

1. Present claim 1 discloses a membrane which, however, is partly defined by the process of its manufacture ("product-by-process"). Due to the selected definition it is not unambiguously clear which of the mentioned features are in fact to be found in the final product (Art. 6 PCT). For instance the general expression "prepared from" (in some contracting states considered merely to read "obtainable from") in principle allows for all characteristics listed to disappear, in particular as the process steps, the various ratios between and specific nature of monomers involved as well as the reaction conditions have not been defined. Claim 34 is subject to the same deficiencies.

1.1 Moreover, claim 1 refers to a polymer to be used in the preparation of the coated membrane. However, it is stated that the polymer comprises various monomers such as "unsaturated monomers". It is not clear (Art. 6 PCT) whether the polymer is originally formed from such monomers - which then no longer constitutes monomers but rather units of the polymer, - whether the coating of the membrane is in fact made from the listed monomers rather from a polymer; and in particular whether the crosslinked coating/polymer will still contain "unsaturated" monomers and/or units [see e.g. page 7, lines 24-27; page 8, lines 8-12]].

1.2 From the wording of claim 1 (27 and 28, thus 34) it is In fact not even clear (Art. 6 PCT) whether the mentioned polymer comprises all three types of monomers, or whether the coating is prepared from a polymer comprising one type of polymerized monomer and (then crosslinked with) the two other types of monomers being (up till crosslinking) actually in their monomeric form [see for instance page 6, lines 9-14].

1.3 The applicant has argued that the polymer coating according to the invention comprises a polymer made from the mentioned three classes of monomers. Although this is not necessarily found to correspond to the subject matter actually claimed (in particular in respect of claim 22), it has for the purpose of the present report been assumed that the crosslinked coating defined in/via independent claims 1, 27 and 28 (and thus 34) comprises a crosslinked polymer which is formed from units carrying a negatively charged group, units carrying a non-ionic

hydrophilic group as well as units comprising one or more of the mentioned acrylamides; and wherein said polymer (due to crosslinking with acrylamide) essentially does not comprise any unsaturated/monomeric groups.

1.4 The subject matter of claim 7 is rendered unclear (Art. 6 PCT) by the reference to a nonionic monomer in claim 2 which is then further defined as an acrylate. The acrylate functionality (propenoate) is considered to be ionic.

1.5 Claim 20 refer to the presence of an initiator in the composition from which the coating is produced. However, it is not clear (Art. 6 PCT) how such presence is unambiguously deducible from the end product which is claimed per se.

1.6 It is not clear what is meant by "substrate polymer" in claims 23 (Art. 6 PCT). It is assumed that it merely means the substrate contains polymeric material.

1.7 Claim 35 is related to a device comprising the membrane according to any of claims 1-26 and 34. However, as no further features characterize the "device" it is not clear whether said claim is actually limited over said claims. The claim is thus redundant.

1.8 Claims 27 and 28 (and 1 as mentioned above) define processes in which step (b) is contacting the substrate with a polymer composition comprising unsaturated monomers. This apparent contradiction renders the subject matter of the claims unclear (Art. 6 PCT).

1.9 Furthermore, claims 27 and 28 comprise the optional step of extracting residue from the membrane. It is, however, not clear which residue is addressed and to which extent said residue is to be extracted. In any event said step is optional and thus superfluous.

1.10 It is not clear from the expression "hydroxyl-rich" used in claim 4 how many hydroxyl-groups must be present in the stated material (Art. 6 PCT). The expression appears to be subjective and thus not suited for defining the subject matter for which protection is sought.

From the description it emanates that the definition should be two or more hydroxyl groups per molecule [passage bridging pages 6 and 7]. However, as said claim merely states that the coating (the crosslinked polymer?) contains such "material" in no particular amount/ratio, it is not clear whether this feature actually limits the subject matter at all (Art. 6 PCT).

1.11 It is not clear what is to be considered as a "biomolecule" as defined in claims 37. The subject matter of said claim is thus unclear (Art. 6 PCT).

1.12 The obscurities addressed above in respect of claims 1, 27 and 28, the subject matter of "product-by-process" claim 34 is rendered equally unclear (Art. 6 PCT). In fact the subject matter of said claim (or claim 1) is considered essentially redundant. It should be noted that in some contracting states two or more claims to the same subject matter is not accepted.

1.13 In respect of claim 22 it is - in view of the above discussion and the description e.g. page 8, lines 8-10 - not entirely clear (Art. 6 PCT) whether the so-called crosslinks will in fact be "traditional" crosslinks between separate polymeric chains or rather be bonds within a (co-)polymerized network.

1.14 In view of the limited number of examples present in the application it is found questionable whether the very broadly defined scope of the claims has sufficient support (Art. 5 and 6 PCT). It is found unlikely that any combination of monomers claimed will in fact lead to a membrane applicable for the intended purpose.

Re Item V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US-A-5 021 160 (S.M. WOLPERT) 4 June 1991 (1991-06-04)
D2: WO 98 17377 A (MC MASTER UNIVERSITY) 30 April 1998 (1998-04-30)
D3: EP-A-0 474 617 (MONSANTO COMPANY) 11 March 1992 (1992-03-11)

D4: US-A-5 843 789 (H. NOMURA) 1 December 1998 (1998-12-01)
D5: US-A-4 455 370 (B.W. BARTELSMAN) 19 June 1984 (1984-06-19)
D6: US-A-5 783 094 (M.A. KRAUS) 21 July 1998 (1998-07-21)

1. The present invention relates in general to negatively charged microporous membranes comprising a substrate and a crosslinked coating providing a negative charge, the process of manufacturing such membranes as well as their use. New product claim 1 as well as process claim 27 (and in principle claim 28) involve the use of three monomers in the formation of the crosslinked coating.

1.1 Negatively charged microporous membranes comprising a substrate and a crosslinked coating having a negative charge are obviously known in the art of membranes [vide e.g. D1 column 5, lines 7-10]. D1, furthermore, discloses the use of various monomers such as N-methylolacrylamide (IBMA), methacrylates (HEMA) and monomers comprising a sulphonic acid moiety (AMPS) [vide the abstract and claims 1-5] as well as the use of polysulphone containing substrates [claims 15-16] and initiator for the polymerisation [The examples].

1.2 D1 apparently does not discloses the combined use of all three types of monomers, but merely states that AMPS (monomers/polymers) advantageously can be crosslinked using IBMA or HEMA.

1.3 Nevertheless, it is considered that a person skilled in the art reading D1 would readily realise that AMPS could be crosslinked using both IBMA and HEMA.

1.4 As no particular effect of using both types of crosslinkers can be derived from the application, or any information which would lead a skilled person to believe that the use of more than one type of crosslinkers would be detrimental is evident, the subject matter of claims 1 and 27 is considered obvious (Art. 33(3) PCT).

1.5 By the same token the subject matter of claims 2-4, 6-11, 16-18, 20-21, 29-35 is considered obvious. The subject matter of claims 22-26 will as an inherent consequence also be obvious (Art. 33(3) PCT).

2. Membranes of the present type are known to have negative charges (i.e. in terms of acid residues) [D1 column 4, lines 24-26]. The use of a carboxy-functionalised acrylamide is considered an obvious alternative to sulphones in view of the limited number of possible choices. Moreover, no surprising technical effect can be derived from the use of such monomers over the use of sulphones. Accordingly the subject matter of claims 12-15 cannot be seen to involve an inventive step (Art. 33(3) PCT).

3. Claims 5, 19 and 28 relate to the use of polysaccharides essentially in terms of polysaccharides such as dextran in order to introduce non-ionic polar groups, from the description page 7, line 13 it is clear that any suitable polysaccharide may be used.

3.1 The general application of polysaccharides is known from D3 which likewise discloses negatively charged microporous membranes comprising a substrate and a crosslinked coating providing a negative charge. D3 teaches i.a. the use of e.g. hydroxypropylcellulose [page 4, lines 9-11] in the monomer composition making up the coating of the membrane substrate. As no particular advantage of using dextran over any other polysaccharide (e.g. in terms of hydroxypropylcellulose mentioned in D3) can be derived from the application, the selection of dextran is considered to be equally obvious as the selection of any other such material. Accordingly it is considered that claims 5, 19 and 28 lack an inventive step (Art. 33(3) PCT).

3.2 It is also noted that polysaccharides, i.a. dextran, is known for use in membrane coatings and within the field of filtering liquids comprising biological compounds [D6, column 2, lines 11-35].

4. With respect to the use of the membranes according to the invention for transferring certain "biomolecules" from an electrophoresis gel as defined in claims 36-40, it appears that use of microporous membranes for this purpose is known [vide D5 column 2, lines 30-38]. Although documents D1-D3 do not explicitly mention that the membranes can be used to separate "biomolecules" from an electrophoresis gel, such use is considered to be obvious (Art. 33(3) PCT) taking the properties of the membranes into consideration. D1, for instance

suggests the filtration of aqueous liquids including biological or parenteral liquids or the use for plasmapheresis.

5. Also D2 appears to constitute pertinent prior art, although apparently not disclosing the use of the three types of monomers. D2 discloses charged membranes comprising a porous substrate and a porous in-situ crosslinked polyelectrolyte or hydrogel disposed on said substrate [the abstract]. As monomers suitable for use according to D2 e.g. acrylic acid and acrylamido-alkyl-sulphonic acid is mentioned [page 5, lines 11-14]. The membranes according to D2 can be used in e.g. dialysis and electrodialysis [page 1, line 25]

6. D4 discloses membranes composed by a porous substrate of e.g. polysulphone onto which a composition comprising monomers e.g. olefinic carboxylic acid is in-situ plasma-polymerised [the abstract, column 6, lines 40-50; column 7, lines 7-11]. In the broadest sense of the term "coating" it would appear to also comprise such plasma treatment as mentioned in D4.

7. In the description the applicant states that amide-amide crosslinks occur between two acrylamide crosslinking agents and that the amide-ester crosslinks occur between one acrylamide crosslinking agent and the "nonionic hydrophilic monomer" (for instance being a hydroxy or alkoxy acrylic monomer) [page 8, lines 13-28; page 6, lines 2-3]. It would thus appear that amide-ester "crosslinks" at least in theory could also occur between two acrylamide crosslinking agents of the preferred art (N-hydroxy- or N-alkoxy-acrylamide).

7.1 In that case the subject matter of claims 22-26 even lacks novelty over the inherent disclosure of D1 (Art. 33(2) PCT).

8. Nevertheless, it would appear that novelty as well as inventive step for specific embodiments of the present invention for which a surprising effect could be demonstrated e.g. in terms of comparative experimental data (relative to D1-D3) could be acknowledgeable in view of the cited prior art.

9. Industrial applicability is self-evident for the subject matter of all claims (Art. 33(4) PCT).

Re Item IV Lack of unity of invention

1. Considering the objections raised under above section V with regard to inventive step it appears that the application relates to several embodiments of the overall invention not so linked as to provide a common both novel and inventive concept (Art. 3(4)(iii) and R. 13 PCT).

1.1 For instance the only common mandatory features of claims 1 and 22 are a membrane with negatively charged polymeric coating. Such membranes are, however, known.

1.2 Likewise the common novel and inventive concept linking independent process claims 27 and 28 is far from being apparent.

Re Item VII Certain defects in the international application

1. Despite the amendments made to the description, it is not in conformity with the claims as required by Rule 5.1(a)(iii) PCT.

high dynamic binding capacity and selectivity for biomolecules. There further exists a need for a membrane that has low non-specific binding or low binding that results from hydrophobic interactions. There further exists a need for a membrane that can withstand high fluid flow velocities. There further exists a need for a membrane that involves preparation chemistries and/or processes that are not cumbersome to practice.

These advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

U.S. Patent 5,021,160 discloses copolymers synthesized from 2-acrylamido-2-methyl-1-propane sulfonic acid and either N-(isobutoxymethyl) acrylamide or 2-hydroxyethyl methacrylate and a process for preparing anionic charge modified microporous filtration membranes.

WO 98/17377 discloses charged membranes comprising a porous substrate and a cross-linked polyelectrolyte or hydrogel located in the pores of the substrate.

European Patent Application No. 0 474 617 A1 discloses a surface modified support membrane wherein the support membrane has a layer of hydrogel deposited on the surface thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the breakthrough curve for lysozyme obtained on an embodiment membrane of the present invention. The x-axis represents the filtration time, and the y-axis represents the absorbance of the filtrate at 280 nm and is indicative of the concentration of the protein. See Example 2 for additional details.

Fig. 2 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present invention. The x-axis and y-axis are as described in Fig. 1. See Example 3 for additional details.

Fig. 3 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present

invention. The x-axis and y-axis are as described in Fig. 1. See Example 4 for additional details.

BRIEF SUMMARY OF THE INVENTION

Many of the foregoing needs have been fulfilled by the present invention which provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having negatively charged groups. In a preferred embodiment, the membrane can be prepared from a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-

polymerization is carried out for a period of from about 16 hours to about 24 hours. The viscosity of the solution is typically below about 2000 cps (2 Pa.s), e.g., preferably from about 50 cps (0.05 Pa.s) to about 500 cps (0.5 Pa.s), and more preferably from about 100 cps (0.1 Pa.s) to about 500 cps (0.5 Pa.s). According to certain embodiments, the viscosity is from about 100 cps (0.1 Pa.s) to about 250 cps (0.25 Pa.s).

The polymerization solution can contain the anionic acrylic monomer (A), the crosslinking agent (B), and the non-ionic hydrophilic monomer (C) in a suitable ratio. The percentage of each monomer (A, B, or C) can be from about 0.1 to 30% by weight, preferably from about 0.1 to 20% by weight.

It is believed that the crosslinked coating comprises amide-ester crosslinks that form as a result of the reaction of the nonionic hydrophilic monomer with the crosslinking agent. For example, these bonds form as a result of the reaction of the hydroxyl groups in the hydroxyalkyl acrylate with the N-(isobutoxymethyl)- acrylamide. In addition, amide-amide crosslinks also form as a result of the reaction between two N-(isobutoxymethyl)acrylamide monomers. For example, the amide-ester crosslink can have the formula:

$$-C(=O)O-R-NH-C(=O),$$

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably $-CH_2-CH_2-CH_2-O-CH_2-$. The amide-amide crosslink can have the formula:

$$-C(=O)NH-R-NH-C(=O),$$

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably $-CH_2-O-CH_2-$.

The coating solution contains the anionic polymer prepared as above and, optionally, a polysaccharide, preferably a dextran. The anionic polymer and the polysaccharide can be present in the coating solution in the ratio of from about 100:1 to about 1:100, preferably from about 10:1 to about 1:10, and more preferably from about 5:1 to about 1:5.

The coating solution contains the anionic polymer and, optionally dextran, in an amount of from about 0.01% to about

for example, the membrane preferably has a water flow rate above 5 mL/min/cm^2, and preferably above 10 mL/min/cm^2, e.g., from about 20 mL/min/cm^2 to about 160 mL/min/cm^2, and preferably from about 25 mL/min/cm^2 to about 100 mL/min/cm^2 at 24 inch Hg. The membrane is robust and can withstand high treatment fluid flow rates. Thus, the membrane can be subjected to flow rates up to 10 cm/min, for example, from about 1 cm/min to 10 cm/min at 10 psi (68.9 kPa). The membrane has an open water bubble point of below about 70 psi (482 kPa), e.g., from about 2.5 psi (9.39 kPa) to about 70 psi (482 kPa), and preferably from about 5 psi (34.47 kPa) to about 50 psi (344.7 kPa).

The membrane of the present invention has a high charge density. The charge density of the membrane can be measured by methods known to those of ordinary skill in the art. For example, the charge density can be measured by the membrane's ability to bind a positively charged dye. Illustratively, the membrane has a Methylene Blue dye binding capacity of at least about 10 mL, e.g., from about 10 mL to about 1000 mL, and preferably from about 100 mL to about 800 mL, when tested with a 10 ppm dye solution in water. Methylene Blue is a positively charged dye. The dye binding capacity is measured by filtering under a 24 inch Hg negative pressure, a 10 ppm by weight solution, pH 6.6, of Methylene Blue dye in a membrane disc of 25 mm diameter, and monitoring the volume of the filtrate until a trace of the dye begins to appear in the filtrate.

The membrane of the present invention has a high specific protein binding capacity. The membrane has a lysozyme specific binding capacity of above 10 mg/mL, e.g., from about 10 mg/mL to about 130 mg/mL and preferably from about 25 mg/mL to about 120 mg/mL. The specific binding capacity can be determined by the following illustrative method. A fluid containing a lysozyme protein in 10 mM MES buffer, pH 5.5, is filtered by passing through a membrane at 1 cm/min and the concentration of the protein in the filtrate is measured as a function of time. The concentration of the protein can be

determined spectrophotometrically, e.g., by measuring the

stack can be eluted under a gradient - 7 ml from 10 mM MES buffer at a pH of 5.5 to 1M NaCl-10 mM MES buffer at a pH of 5.5. The flow rate can be 4 ml/min. Cytochrome C elutes first, followed by lysozyme.

The following examples further illustrate the present invention but should not be construed in any way limiting the scope of the invention.

EXAMPLE 1

This Example illustrates a method of preparing a polymer composition for preparing an embodiment of the negatively charged membrane of the present invention.

2-Acrylamido-2-methyl-1-propanesulfonic acid, N-(isobutoxymethyl)acrylamide, and hydroxypropyl methacrylate were combined in a weight ratio of 8.0:2.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 12% by weight. Ammonium persulfate was used as the initiator at 0.3% by weight of the solution. The polymerization was carried out for a period of about 10-15 hours at ambient temperature (20-25°C). The resulting solution had a viscosity of 166 cps (0.166 Pa.s).

EXAMPLE 2

This Example illustrates a method for preparing an embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

A coating solution was prepared by mixing the polymerization solution described in Example 1 and a water solution of dextran, molecular weight 148 K, so that the resulting solution contains polymer and dextran in the weight ratio of 3:1.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 µm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI

first 10 minutes of the treatment confirmed that the membrane did not leak. The nearly vertical slope indicates that the membrane was capable of providing high resolution.

EXAMPLE 4

This Example illustrates a method for preparing another embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

2-Acrylamidoglycolic acid, 2-carboxyethyl acrylate, N-(isobutoxymethyl)acrylamide, N-(hydroxymethyl)-acrylamide, and hydroxypropyl acrylate were combined in a weight ratio of 5.0:5.0:3.0:1.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 16% by weight. Ammonium persulfate was used as the initiator at 0.4% by weight of the solution. The polymerization was carried out for a period of about 16-24 hours at ambient temperature. The resulting solution had a viscosity of 116 cps (0.116 Pa.s). A coating solution was prepared by mixing the polymerization solution and a water solution of dextran, molecular weight 148 K, so that the resulting solution contained 4% polymer and 1.33% dextran by weight.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 µm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI water for 1 hour. The resulting membrane was dried in an oven to obtain another embodiment of the present invention.

The membrane obtained above was tested with a solution containing lysozyme. The solution was contained 213.6 µg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 3. The membrane had a lysozyme binding capacity of 45 mg/ml. The relatively flat curve obtained during the first 10 minutes of the treatment confirmed that the membrane

WHAT IS CLAIMED IS:

1. A negatively charged microporous membrane comprising a porous substrate and a crosslinked coating, wherein the crosslinked coating is prepared from a polymer comprising an unsaturated monomer having a negatively charged group, a hydrophilic non-ionic unsaturated monomer, and at least one or more N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide.

2. The negatively charged microporous membrane of claim 1, wherein the hydrophilic non-ionic unsaturated monomer is an acrylic monomer.

3. The negatively charged microporous membrane of claim 1 or 2, wherein the N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide includes an alkyl group of 4 carbon atoms or less.

4. The negatively charged microporous membrane of any of claims 1-3, wherein the crosslinked coating includes a hydroxyl-rich material.

5. The negatively charged microporous membrane of claim 4, wherein the hydroxyl-rich material is a polysaccharide.

6. The negatively charged microporous membrane of any of claims 1-5, wherein said negatively charged group is a sulfonic or carboxylic acid.

7. The negatively charged microporous membrane of claim 2, wherein said acrylic monomer is an acrylate or acrylamide.

8. The negatively charged microporous membrane of claim 7, wherein said acrylic monomer is an acrylamide.

9. The negatively charged microporous membrane of claim 8, wherein said acrylamide is an alkylacrylamide.

10. The negatively charged microporous membrane of claim 9, wherein said acrylamide has a sulfonic acid group.

11. The negatively charged microporous membrane of claim 10, wherein said acrylamide is acrylamido-N-alkylsulfonic acid.

12. The negatively charged microporous membrane of claim 9, wherein said alkylacrylamide has a carboxylic acid group.

13. The negatively charged microporous membrane of claim 12, wherein said polymer includes a further acrylic monomer having a carboxylic acid group.

14. The negatively charged microporous membrane of claim 13, wherein said further acrylic monomer is an acrylate.

15. The negatively charged microporous membrane of claim 14, wherein said acrylate is β-carboxyethyl acrylate.

16. The negatively charged microporous membrane of claim 4, wherein said acrylic monomer is a hydroxyacrylic monomer.

17. The negatively charged microporous membrane of claim 16, wherein said hydroxyacrylic monomer is a hydroxyacrylamide or an hydroxyacrylate.

18. The negatively charged microporous membrane of any of claims 1-17, wherein said polymer includes an N-(alkoxymethyl)acrylamide.

19. The negatively charged microporous membrane of claim 5, wherein said polysaccharide is dextran.

20. The negatively charged microporous membrane of claim 1, wherein the polymer comprising an unsaturated monomer having a negatively

charged group, a hydrophilic non-ionic unsaturated monomer, and at least one or more N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide is prepared by employing a free radical initiator.

21. The negatively charged microporous membrane of any of claims 1-20 having a dynamic protein binding capacity of about 25 mg/ml lysozyme or more.

22. A negatively charged microporous membrane comprising a porous substrate and a crosslinked coating comprising negatively charged groups and amide-amide and amide-ester crosslinks.

23. The negatively charged microporous membrane of any of claims 1-22, wherein said porous substrate comprises a substrate polymer.

24. The negatively charged microporous membrane of claim 23, wherein said substrate polymer is selected from the group consisting of polyaromatics, polysulfones, polyolefins, polystyrenes, polyamides, polyimides, cellulose acetates, cellulose nitrates, polycarbonates, polyesters, and fluoropolymers.

25. The negatively charged microporous membrane of claim 24, wherein said substrate polymer is a polysulfone.

26. The negatively charged microporous membrane of any of claims 1-25, wherein said porous substrate is hydrophilic.

27. A process for preparing a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having negatively charged groups, the process comprising:

(a) providing a porous substrate;

(b) contacting said substrate with a polymer comprising an unsaturated monomer having a negatively charged group, a hydrophilic non-ionic unsaturated monomer, and at least one or more of a N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide;

(c) curing the substrate obtained in (b) to obtain the negatively charged microporous membrane; and

(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

28. A process for preparing a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having negatively charged groups, the process comprising:

(a) providing a porous substrate;

(b) contacting said substrate with a polysaccharide and a polymer comprising an unsaturated monomer having a negatively charged group and an N-(hydroxymethyl)- or N-(alkoxymethyl)-acrylamide;

(c) curing the substrate obtained in (b) to obtain the negatively charged microporous membrane; and

(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

29. The process of claim 27 or 28, wherein said negatively charged group is a sulfonic or carboxylic acid.

30. The process of any of claims 27-29, wherein said unsaturated monomer having a negatively charged group is an acrylic monomer having a sulfonic or carboxylic acid group.

31. The process of claim 30, wherein said acrylic monomer having a sulfonic or carboxylic acid group is an acrylate or an acrylamide.

32. The process of claim 27, wherein the substrate is contacted in (b) with said polymer and a hydroxyl-rich material.

33. The process of any of claims 27-32, wherein said porous substrate comprises a substrate polymer.

34. The negatively charged microporous membrane prepared by the process of any of claims 27-33.

35. A device comprising the negatively charged microporous membrane of any of claims 1-26 and 34.

36. A process for separating positively charged material from a fluid, said process comprising placing said fluid in contact with the negatively charged microporous membrane of any of claims 1-26 and 34 so as to adsorb the positively charged material to said membrane.

37. The process of claim 36, wherein said positively charged material is a biomolecule.

38. A process for transferring biomolecules from an electrophoresis gel comprising contacting said electrophoresis gel with a membrane of any of claims 1-26 and 34 and transferring the biomolecules to the membrane.

39. The process of claim 38, wherein said biomolecule is selected from the group consisting of proteins, polypeptides, amino acids, and nucleic acids, and combinations thereof.

40. The process of claim 38 or 39, further including recovering the positively charged material adsorbed on the membrane.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
440200/PALL		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 04745	25/02/2000	25/02/1999
Applicant		
PALL CORPORATION		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/04745

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01D67/00 B01D61/00 B01J39/20 B01J47/12 B01J20/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01D B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 021 160 A (S.M. WOLPERT) 4 June 1991 (1991-06-04) the whole document ---	1-5, 9-16, 21-24, 28-35, 37-39, 41-44
X	WO 98 17377 A (MC MASTER UNIVERSITY) 30 April 1998 (1998-04-30) page 5, line 8 - line 14 page 36 --- -/--	1,9,28, 30,31, 34,43

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 July 2000	19/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hilgenga, K

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/US 00/04745

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 474 617 A (MONSANTO COMPANY) 11 March 1992 (1992-03-11)	1,6,7,9, 28, 30-34, 43,44
A	page 5, line 40 - line 45; claims 9,15	26
A	US 5 843 789 A (H. NOMURA) 1 December 1998 (1998-12-01) column 6, line 44 column 10, line 51 - line 65	1,9,11, 28, 30-34, 43,44
A	US 4 455 370 A (B.W. BARTELSMAN) 19 June 1984 (1984-06-19) claim 1	45,46
A	US 5 783 094 A (M.A. KRAUS) 21 July 1998 (1998-07-21)	
A	US 4 617 321 A (R.J. MACDONALD) 14 October 1986 (1986-10-14)	
A	DE 44 39 444 A (MERCK PATENT) 9 May 1996 (1996-05-09)	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat[illegible] Application No
PCT/US 00/04745

Patent document cited in search report	Publication date	Patent family member(s)	Publication dat
US 5021160 A	04-06-1991	WO 9110498 A	25-07-1991
WO 9817377 A	30-04-1998	AU 4613097 A	15-05-1998
		EP 0948392 A	13-10-1999
		JP 2000503898 T	04-04-2000
EP 474617 A	11-03-1992	US 5104729 A	14-04-1992
		AU 633449 B	28-01-1993
		AU 8254891 A	27-02-1992
		CA 2049459 A,C	21-02-1992
		JP 4250832 A	07-09-1992
US 5843789 A	01-12-1998	AU 5737596 A	29-11-1996
		WO 9636877 A	21-11-1996
US 4455370 A	19-06-1984	NONE	
US 5783094 A	21-07-1998	US 5895575 A	20-04-1999
		AU 5309396 A	30-10-1996
		EP 0869835 A	14-10-1998
		WO 9632178 A	17-10-1996
US 4617321 A	14-10-1986	NONE	
DE 4439444 A	09-05-1996	WO 9614151 A	17-05-1996
		EP 0789620 A	20-08-1997
		JP 10508249 T	18-08-1998

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

JAY, Jeremy M. et al
LEYDIG, VOIT & MAYER
700 Thirteenth Street, N.W.
Suite 300
Washington, D.C. 20005
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	25.05.2001
Applicant's or agent's file reference 440200/PALL	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04745	25/02/2000	25/02/1999

Applicant
PALL CORPORATION et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Ipinazar, P Tel.+49 89 2399-8131

Form PCT/IPEA/416 (July 1992)

TENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
440200/PALL		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04745	25/02/2000	25/02/1999
International Patent Classification (IPC) or national classification and IPC B01D67/00		
Applicant PALL CORPORATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 13 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/09/2000	25.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Nissen, V Telephone No. +49 89 2399 8619

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/04745

I. Basis of th report

1. With regard to the **el ments** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-20 as originally filed

Claims, No.:

1-47 as originally filed

Drawings, sheets:

1-3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21, 27-40
	No:	Claims	22-26
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-40

Industrial applicability (IA)	Yes:	Claims	1-40
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item VIII Certain obs rvations on the international applicati n

1. Present claim 1 discloses a membrane which, however, is partly defined by the process of its manufacture ("product-by-process"). Due to the selected definition it is not unambiguously clear which of the mentioned features are in fact to be found in the final product (Art. 6 PCT). For instance the general expression "prepared from" (in some contracting states considered merely to read "obtainable from") in principle allows for all characteristics listed to disappear, in particular as the process steps, the various ratios between and specific nature of monomers involved as well as the reaction conditions have not been defined. Claim 34 is subject to the same deficiencies.

1.1 Moreover, claim 1 refers to a polymer to be used in the preparation of the coated membrane. However, it is stated that the polymer comprises various monomers such as "unsaturated monomers". It is not clear (Art. 6 PCT) whether the polymer is originally formed from such monomers - which then no longer constitutes monomers but rather units of the polymer, - whether the coating of the membrane is in fact made from the listed monomers rather from a polymer; and in particular whether the crosslinked coating/polymer will still contain "unsaturated" monomers and/or units [see e.g. page 7, lines 24-27; page 8, lines 8-12]].

1.2 From the wording of claim 1 (27 and 28, thus 34) it is In fact not even clear (Art. 6 PCT) whether the mentioned polymer comprises all three types of monomers, or whether the coating is prepared from a polymer comprising one type of polymerized monomer and (then crosslinked with) the two other types of monomers being (up till crosslinking) actually in their monomeric form [see for instance page 6, lines 9-14].

1.3 The applicant has argued that the polymer coating according to the invention comprises a polymer made from the mentioned three classes of monomers. Although this is not necessarily found to correspond to the subject matter actually claimed (in particular in respect of claim 22), it has for the purpose of the present report been assumed that the crosslinked coating defined in/via independent claims 1, 27 and 28 (and thus 34) comprises a crosslinked polymer which is formed from units carrying a negatively charged group, units carrying a non-ionic

hydrophilic group as well as units comprising one or more of the mentioned acrylamides; and wherein said polymer (due to crosslinking with acrylamide) essentially does not comprise any unsaturated/monomeric groups.

1.4 The subject matter of claim 7 is rendered unclear (Art. 6 PCT) by the reference to a nonionic monomer in claim 2 which is then further defined as an acrylate. The acrylate functionality (propenoate) is considered to be ionic.

1.5 Claim 20 refer to the presence of an initiator in the composition from which the coating is produced. However, it is not clear (Art. 6 PCT) how such presence is unambiguously deducible from the end product which is claimed per se.

1.6 It is not clear what is meant by "substrate polymer" in claims 23 (Art. 6 PCT). It is assumed that it merely means the substrate contains polymeric material.

1.7 Claim 35 is related to a device comprising the membrane according to any of claims 1-26 and 34. However, as no further features characterize the "device" it is not clear whether said claim is actually limited over said claims. The claim is thus redundant.

1.8 Claims 27 and 28 (and 1 as mentioned above) define processes in which step (b) is contacting the substrate with a polymer composition comprising unsaturated monomers. This apparent contradiction renders the subject matter of the claims unclear (Art. 6 PCT).

1.9 Furthermore, claims 27 and 28 comprise the optional step of extracting residue from the membrane. It is, however, not clear which residue is addressed and to which extent said residue is to be extracted. In any event said step is optional and thus superfluous.

1.10 It is not clear from the expression "hydroxyl-rich" used in claim 4 how many hydroxyl-groups must be present in the stated material (Art. 6 PCT). The expression appears to be subjective and thus not suited for defining the subject matter for which protection is sought.

From the description it emanates that the definition should be two or more hydroxyl groups per molecule [passage bridging pages 6 and 7]. However, as said claim merely states that the coating (the crosslinked polymer?) contains such "material" in no particular amount/ratio, it is not clear whether this feature actually limits the subject matter at all (Art. 6 PCT).

1.11 It is not clear what is to be considered as a "biomolecule" as defined in claims 37. The subject matter of said claim is thus unclear (Art. 6 PCT).

1.12 The obscurities addressed above in respect of claims 1, 27 and 28, the subject matter of "product-by-process" claim 34 is rendered equally unclear (Art. 6 PCT). In fact the subject matter of said claim (or claim 1) is considered essentially redundant. It should be noted that in some contracting states two or more claims to the same subject matter is not accepted.

1.13 In respect of claim 22 it is - in view of the above discussion and the description e.g. page 8, lines 8-10 - not entirely clear (Art. 6 PCT) whether the so-called crosslinks will in fact be "traditional" crosslinks between separate polymeric chains or rather be bonds within a (co-)polymerized network.

1.14 In view of the limited number of examples present in the application it is found questionable whether the very broadly defined scope of the claims has sufficient support (Art. 5 and 6 PCT). It is found unlikely that any combination of monomers claimed will in fact lead to a membrane applicable for the intended purpose.

Re Item V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US-A-5 021 160 (S.M. WOLPERT) 4 June 1991 (1991-06-04)
D2: WO 98 17377 A (MC MASTER UNIVERSITY) 30 April 1998 (1998-04-30)
D3: EP-A-0 474 617 (MONSANTO COMPANY) 11 March 1992 (1992-03-11)

D4: US-A-5 843 789 (H. NOMURA) 1 December 1998 (1998-12-01)
D5: US-A-4 455 370 (B.W. BARTELSMAN) 19 June 1984 (1984-06-19)
D6: US-A-5 783 094 (M.A. KRAUS) 21 July 1998 (1998-07-21)

1. The present invention relates in general to negatively charged microporous membranes comprising a substrate and a crosslinked coating providing a negative charge, the process of manufacturing such membranes as well as their use. New product claim 1 as well as process claim 27 (and in principle claim 28) involve the use of three monomers in the formation of the crosslinked coating.

1.1 Negatively charged microporous membranes comprising a substrate and a crosslinked coating having a negative charge are obviously known in the art of membranes [vide e.g. D1 column 5, lines 7-10]. D1, furthermore, discloses the use of various monomers such as N-methylolacrylamide (IBMA), methacrylates (HEMA) and monomers comprising a sulphonic acid moiety (AMPS) [vide the abstract and claims 1-5] as well as the use of polysulphone containing substrates [claims 15-16] and initiator for the polymerisation [The examples].

1.2 D1 apparently does not discloses the combined use of all three types of monomers, but merely states that AMPS (monomers/polymers) advantageously can be crosslinked using IBMA or HEMA.

1.3 Nevertheless, it is considered that a person skilled in the art reading D1 would readily realise that AMPS could be crosslinked using both IBMA and HEMA.

1.4 As no particular effect of using both types of crosslinkers can be derived from the application, or any information which would lead a skilled person to believe that the use of more than one type of crosslinkers would be detrimental is evident, the subject matter of claims 1 and 27 is considered obvious (Art. 33(3) PCT).

1.5 By the same token the subject matter of claims 2-4, 6-11, 16-18, 20-21, 29-35 is considered obvious. The subject matter of claims 22-26 will as an inherent consequence also be obvious (Art. 33(3) PCT).

2. Membranes of the present type are known to have negative charges (i.e. in terms of acid residues) [D1 column 4, lines 24-26]. The use of a carboxy-functionalised acrylamide is considered an obvious alternative to sulphones in view of the limited number of possible choices. Moreover, no surprising technical effect can be derived from the use of such monomers over the use of sulphones. Accordingly the subject matter of claims 12-15 cannot be seen to involve an inventive step (Art. 33(3) PCT).

3. Claims 5, 19 and 28 relate to the use of polysaccharides essentially in terms of polysaccharides such as dextran in order to introduce non-ionic polar groups, from the description page 7, line 13 it is clear that any suitable polysaccharide may be used.

3.1 The general application of polysaccharides is known from D3 which likewise discloses negatively charged microporous membranes comprising a substrate and a crosslinked coating providing a negative charge. D3 teaches i.a. the use of e.g. hydroxypropylcellulose [page 4, lines 9-11] in the monomer composition making up the coating of the membrane substrate. As no particular advantage of using dextran over any other polysaccharide (e.g. in terms of hydroxypropylcellulose mentioned in D3) can be derived from the application, the selection of dextran is considered to be equally obvious as the selection of any other such material. Accordingly it is considered that claims 5, 19 and 28 lack an inventive step (Art. 33(3) PCT).

3.2 It is also noted that polysaccharides, i.a. dextran, is known for use in membrane coatings and within the field of filtering liquids comprising biological compounds [D6, column 2, lines 11-35].

4. With respect to the use of the membranes according to the invention for transferring certain "biomolecules" from an electrophoresis gel as defined in claims 36-40, it appears that use of microporous membranes for this purpose is known [vide D5 column 2, lines 30-38]. Although documents D1-D3 do not explicitly mention that the membranes can be used to separate "biomolecules" from an electrophoresis gel, such use is considered to be obvious (Art. 33(3) PCT) taking the properties of the membranes into consideration. D1, for instance

suggests the filtration of aqueous liquids including biological or parenteral liquids or the use for plasmapheresis.

5. Also D2 appears to constitute pertinent prior art, although apparently not disclosing the use of the three types of monomers. D2 discloses charged membranes comprising a porous substrate and a porous in-situ crosslinked polyelectrolyte or hydrogel disposed on said substrate [the abstract]. As monomers suitable for use according to D2 e.g. acrylic acid and acrylamido-alkyl-sulphonic acid is mentioned [page 5, lines 11-14]. The membranes according to D2 can be used in e.g. dialysis and electrodialysis [page 1, line 25]

6. D4 discloses membranes composed by a porous substrate of e.g. polysulphone onto which a composition comprising monomers e.g. olefinic carboxylic acid is in-situ plasma-polymerised [the abstract, column 6, lines 40-50; column 7, lines 7-11]. In the broadest sense of the term "coating" it would appear to also comprise such plasma treatment as mentioned in D4.

7. In the description the applicant states that amide-amide crosslinks occur between two acrylamide crosslinking agents and that the amide-ester crosslinks occur between one acrylamide crosslinking agent and the "nonionic hydrophilic monomer" (for instance being a hydroxy or alkoxy acrylic monomer) [page 8, lines 13-28; page 6, lines 2-3]. It would thus appear that amide-ester "crosslinks" at least in theory could also occur between two acrylamide crosslinking agents of the preferred art (N-hydroxy- or N-alkoxy-acrylamide).

7.1 In that case the subject matter of claims 22-26 even lacks novelty over the inherent disclosure of D1 (Art. 33(2) PCT).

8. Nevertheless, it would appear that novelty as well as inventive step for specific embodiments of the present invention for which a surprising effect could be demonstrated e.g. in terms of comparative experimental data (relative to D1-D3) could be acknowledgeable in view of the cited prior art.

9. Industrial applicability is self-evident for the subject matter of all claims (Art. 33(4) PCT).

Re Item IV Lack of unity of invention

1. Considering the objections raised under above section V with regard to inventive step it appears that the application relates to several embodiments of the overall invention not so linked as to provide a common both novel and inventive concept (Art. 3(4)(iii) and R. 13 PCT).

1.1 For instance the only common mandatory features of claims 1 and 22 are a membrane with negatively charged polymeric coating. Such membranes are, however, known.

1.2 Likewise the common novel and inventive concept linking independent process claims 27 and 28 is far from being apparent.

Re Item VII Certain defects in the international application

1. Despite the amendments made to the description, it is not in conformity with the claims as required by Rule 5.1(a)(iii) PCT.

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
440200/PALL		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04745	25/02/2000	25/02/1999
International Patent Classification (IPC) or national classification and IPC B01D67/00		
Applicant PALL CORPORATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 13 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/09/2000	25.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Nissen, V Telephone No. +49 89 2399 8619

Form PCT/IPEA/409 (cover sheet) (January 1994)

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

1,3-7,9-13,15,16, 18,20	as originally filed	
2,2a,8,8a,14,14a, 17,19	with telefax of	28/02/2001

Claims, No.:

41-47	as originally filed	
1-40	with telefax of	28/02/2001

Drawings, sheets:

1-3	as originally filed	

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under Item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21, 27-40
	No:	Claims	22-26
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-40

Industrial applicability (IA) Yes: Claims 1-40
No: Claims

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item VIII Certain observations on the international application

1. Present claim 1 discloses a membrane which, however, is partly defined by the process of its manufacture ("product-by-process"). Due to the selected definition it is not unambiguously clear which of the mentioned features are in fact to be found in the final product (Art. 6 PCT). For instance the general expression "prepared from" (in some contracting states considered merely to read "obtainable from") in principle allows for all characteristics listed to disappear, in particular as the process steps, the various ratios between and specific nature of monomers involved as well as the reaction conditions have not been defined. Claim 34 is subject to the same deficiencies.

1.1 Moreover, claim 1 refers to a polymer to be used in the preparation of the coated membrane. However, it is stated that the polymer comprises various monomers such as "unsaturated monomers". It is not clear (Art. 6 PCT) whether the polymer is originally formed from such monomers - which then no longer constitutes monomers but rather units of the polymer, - whether the coating of the membrane is in fact made from the listed monomers rather from a polymer; and in particular whether the crosslinked coating/polymer will still contain "unsaturated" monomers and/or units [see e.g. page 7, lines 24-27; page 8, lines 8-12]].

1.2 From the wording of claim 1 (27 and 28, thus 34) it is In fact not even clear (Art. 6 PCT) whether the mentioned polymer comprises all three types of monomers, or whether the coating is prepared from a polymer comprising one type of polymerized monomer and (then crosslinked with) the two other types of monomers being (up till crosslinking) actually in their monomeric form [see for instance page 6, lines 9-14].

1.3 The applicant has argued that the polymer coating according to the invention comprises a polymer made from the mentioned three classes of monomers. Although this is not necessarily found to correspond to the subject matter actually claimed (in particular in respect of claim 22), it has for the purpose of the present report been assumed that the crosslinked coating defined in/via independent claims 1, 27 and 28 (and thus 34) comprises a crosslinked polymer which is formed from units carrying a negatively charged group, units carrying a non-ionic

hydrophilic group as well as units comprising one or more of the mentioned acrylamides; and wherein said polymer (due to crosslinking with acrylamide) essentially does not comprise any unsaturated/monomeric groups.

1.4 The subject matter of claim 7 is rendered unclear (Art. 6 PCT) by the reference to a nonionic monomer in claim 2 which is then further defined as an acrylate. The acrylate functionality (propenoate) is considered to be ionic.

1.5 Claim 20 refer to the presence of an initiator in the composition from which the coating is produced. However, it is not clear (Art. 6 PCT) how such presence is unambiguously deducible from the end product which is claimed per se.

1.6 It is not clear what is meant by "substrate polymer" in claims 23 (Art. 6 PCT). It is assumed that it merely means the substrate contains polymeric material.

1.7 Claim 35 is related to a device comprising the membrane according to any of claims 1-26 and 34. However, as no further features characterize the "device" it is not clear whether said claim is actually limited over said claims. The claim is thus redundant.

1.8 Claims 27 and 28 (and 1 as mentioned above) define processes in which step (b) is contacting the substrate with a polymer composition comprising unsaturated monomers. This apparent contradiction renders the subject matter of the claims unclear (Art. 6 PCT).

1.9 Furthermore, claims 27 and 28 comprise the optional step of extracting residue from the membrane. It is, however, not clear which residue is addressed and to which extent said residue is to be extracted. In any event said step is optional and thus superfluous.

1.10 It is not clear from the expression "hydroxyl-rich" used in claim 4 how many hydroxyl-groups must be present in the stated material (Art. 6 PCT). The expression appears to be subjective and thus not suited for defining the subject matter for which protection is sought.

From the description it emanates that the definition should be two or more hydroxyl groups per molecule [passage bridging pages 6 and 7]. However, as said claim merely states that the coating (the crosslinked polymer?) contains such "material" in no particular amount/ratio, it is not clear whether this feature actually limits the subject matter at all (Art. 6 PCT).

1.11 It is not clear what is to be considered as a "biomolecule" as defined in claims 37. The subject matter of said claim is thus unclear (Art. 6 PCT).

1.12 The obscurities addressed above in respect of claims 1, 27 and 28, the subject matter of "product-by-process" claim 34 is rendered equally unclear (Art. 6 PCT). In fact the subject matter of said claim (or claim 1) is considered essentially redundant. It should be noted that in some contracting states two or more claims to the same subject matter is not accepted.

1.13 In respect of claim 22 it is - in view of the above discussion and the description e.g. page 8, lines 8-10 - not entirely clear (Art. 6 PCT) whether the so-called crosslinks will in fact be "traditional" crosslinks between separate polymeric chains or rather be bonds within a (co-)polymerized network.

1.14 In view of the limited number of examples present in the application it is found questionable whether the very broadly defined scope of the claims has sufficient support (Art. 5 and 6 PCT). It is found unlikely that any combination of monomers claimed will in fact lead to a membrane applicable for the intended purpose.

Re Item V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US-A-5 021 160 (S.M. WOLPERT) 4 June 1991 (1991-06-04)
D2: WO 98 17377 A (MC MASTER UNIVERSITY) 30 April 1998 (1998-04-30)
D3: EP-A-0 474 617 (MONSANTO COMPANY) 11 March 1992 (1992-03-11)

D4: US-A-5 843 789 (H. NOMURA) 1 December 1998 (1998-12-01)
D5: US-A-4 455 370 (B.W. BARTELSMAN) 19 June 1984 (1984-06-19)
D6: US-A-5 783 094 (M.A. KRAUS) 21 July 1998 (1998-07-21)

1. The present invention relates in general to negatively charged microporous membranes comprising a substrate and a crosslinked coating providing a negative charge, the process of manufacturing such membranes as well as their use. New product claim 1 as well as process claim 27 (and in principle claim 28) involve the use of three monomers in the formation of the crosslinked coating.

1.1 Negatively charged microporous membranes comprising a substrate and a crosslinked coating having a negative charge are obviously known in the art of membranes [vide e.g. D1 column 5, lines 7-10]. D1, furthermore, discloses the use of various monomers such as N-methylolacrylamide (IBMA), methacrylates (HEMA) and monomers comprising a sulphonic acid moiety (AMPS) [vide the abstract and claims 1-5] as well as the use of polysulphone containing substrates [claims 15-16] and initiator for the polymerisation [The examples].

1.2 D1 apparently does not discloses the combined use of all three types of monomers, but merely states that AMPS (monomers/polymers) advantageously can be crosslinked using IBMA or HEMA.

1.3 Nevertheless, it is considered that a person skilled in the art reading D1 would readily realise that AMPS could be crosslinked using both IBMA and HEMA.

1.4 As no particular effect of using both types of crosslinkers can be derived from the application, or any information which would lead a skilled person to believe that the use of more than one type of crosslinkers would be detrimental is evident, the subject matter of claims 1 and 27 is considered obvious (Art. 33(3) PCT).

1.5 By the same token the subject matter of claims 2-4, 6-11, 16-18, 20-21, 29-35 is considered obvious. The subject matter of claims 22-26 will as an inherent consequence also be obvious (Art. 33(3) PCT).

2. Membranes of the present type are known to have negative charges (i.e. in terms of acid residues) [D1 column 4, lines 24-26]. The use of a carboxy-functionalised acrylamide is considered an obvious alternative to sulphones in view of the limited number of possible choices. Moreover, no surprising technical effect can be derived from the use of such monomers over the use of sulphones. Accordingly the subject matter of claims 12-15 cannot be seen to involve an inventive step (Art. 33(3) PCT).

3. Claims 5, 19 and 28 relate to the use of polysaccharides essentially in terms of polysaccharides such as dextran in order to introduce non-ionic polar groups, from the description page 7, line 13 it is clear that any suitable polysaccharide may be used.

3.1 The general application of polysaccharides is known from D3 which likewise discloses negatively charged microporous membranes comprising a substrate and a crosslinked coating providing a negative charge. D3 teaches i.a. the use of e.g. hydroxypropylcellulose [page 4, lines 9-11] in the monomer composition making up the coating of the membrane substrate. As no particular advantage of using dextran over any other polysaccharide (e.g. in terms of hydroxypropylcellulose mentioned in D3) can be derived from the application, the selection of dextran is considered to be equally obvious as the selection of any other such material. Accordingly it is considered that claims 5, 19 and 28 lack an inventive step (Art. 33(3) PCT).

3.2 It is also noted that polysaccharides, i.a. dextran, is known for use in membrane coatings and within the field of filtering liquids comprising biological compounds [D6, column 2, lines 11-35].

4. With respect to the use of the membranes according to the invention for transferring certain "biomolecules" from an electrophoresis gel as defined in claims 36-40, it appears that use of microporous membranes for this purpose is known [vide D5 column 2, lines 30-38]. Although documents D1-D3 do not explicitly mention that the membranes can be used to separate "biomolecules" from an electrophoresis gel, such use is considered to be obvious (Art. 33(3) PCT) taking the properties of the membranes into consideration. D1, for instance

suggests the filtration of aqueous liquids including biological or parenteral liquids or the use for plasmapheresis.

5. Also D2 appears to constitute pertinent prior art, although apparently not disclosing the use of the three types of monomers. D2 discloses charged membranes comprising a porous substrate and a porous in-situ crosslinked polyelectrolyte or hydrogel disposed on said substrate [the abstract]. As monomers suitable for use according to D2 e.g. acrylic acid and acrylamido-alkyl-sulphonic acid is mentioned [page 5, lines 11-14]. The membranes according to D2 can be used in e.g. dialysis and electrodialysis [page 1, line 25]

6. D4 discloses membranes composed by a porous substrate of e.g. polysulphone onto which a composition comprising monomers e.g. olefinic carboxylic acid is in-situ plasma-polymerised [the abstract, column 6, lines 40-50; column 7, lines 7-11]. In the broadest sense of the term "coating" it would appear to also comprise such plasma treatment as mentioned in D4.

7. In the description the applicant states that amide-amide crosslinks occur between two acrylamide crosslinking agents and that the amide-ester crosslinks occur between one acrylamide crosslinking agent and the "nonionic hydrophilic monomer" (for instance being a hydroxy or alkoxy acrylic monomer) [page 8, lines 13-28; page 6, lines 2-3]. It would thus appear that amide-ester "crosslinks" at least in theory could also occur between two acrylamide crosslinking agents of the preferred art (N-hydroxy- or N-alkoxy-acrylamide).

7.1 In that case the subject matter of claims 22-26 even lacks novelty over the inherent disclosure of D1 (Art. 33(2) PCT).

8. Nevertheless, it would appear that novelty as well as inventive step for specific embodiments of the present invention for which a surprising effect could be demonstrated e.g. in terms of comparative experimental data (relative to D1-D3) could be acknowledgeable in view of the cited prior art.

9. Industrial applicability is self-evident for the subject matter of all claims (Art. 33(4) PCT).

Re Item IV Lack of unity of invention

1. Considering the objections raised under above section V with regard to inventive step it appears that the application relates to several embodiments of the overall invention not so linked as to provide a common both novel and inventive concept (Art. 3(4)(iii) and R. 13 PCT).

1.1 For instance the only common mandatory features of claims 1 and 22 are a membrane with negatively charged polymeric coating. Such membranes are, however, known.

1.2 Likewise the common novel and inventive concept linking independent process claims 27 and 28 is far from being apparent.

Re Item VII Certain defects in the international application

1. Despite the amendments made to the description, it is not in conformity with the claims as required by Rule 5.1(a)(iii) PCT.

high dynamic binding capacity and selectivity for biomolecules. There further exists a need for a membrane that has low non-specific binding or low binding that results from hydrophobic interactions. There further exists a need for a membrane that can withstand high fluid flow velocities. There further exists a need for a membrane that involves preparation chemistries and/or processes that are not cumbersome to practice.

These advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

U.S. Patent 5,021,160 discloses copolymers synthesized from 2-acrylamido-2-methyl-1-propane sulfonic acid and either N-(isobutoxymethyl) acrylamide or 2-hydroxyethyl methacrylate and a process for preparing anionic charge modified microporous filtration membranes.

WO 98/17377 discloses charged membranes comprising a porous substrate and a cross-linked polyelectrolyte or hydrogel located in the pores of the substrate.

European Patent Application No. 0 474 617 A1 discloses a surface modified support membrane wherein the support membrane has a layer of hydrogel deposited on the surface thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the breakthrough curve for lysozyme obtained on an embodiment membrane of the present invention. The x-axis represents the filtration time, and the y-axis represents the absorbance of the filtrate at 280 nm and is indicative of the concentration of the protein. See Example 2 for additional details.

Fig. 2 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present invention. The x-axis and y-axis are as described in Fig. 1. See Example 3 for additional details.

Fig. 3 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present

invention. The x-axis and y-axis are as described in Fig. 1. See Example 4 for additional details.

BRIEF SUMMARY OF THE INVENTION

Many of the foregoing needs have been fulfilled by the present invention which provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having negatively charged groups. In a preferred embodiment, the membrane can be prepared from a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-

polymerization is carried out for a period of from about 16 hours to about 24 hours. The viscosity of the solution is typically below about 2000 cps (2 Pa.s), e.g., preferably from about 50 cps (0.05 Pa.s) to about 500 cps (0.5 Pa.s), and more preferably from about 100 cps (0.1 Pa.s) to about 500 cps (0.5 Pa.s). According to certain embodiments, the viscosity is from about 100 cps (0.1 Pa.s) to about 250 cps (0.25 Pa.s).

The polymerization solution can contain the anionic acrylic monomer (A), the crosslinking agent (B), and the non-ionic hydrophilic monomer (C) in a suitable ratio. The percentage of each monomer (A, B, or C) can be from about 0.1 to 30% by weight, preferably from about 0.1 to 20% by weight.

It is believed that the crosslinked coating comprises amide-ester crosslinks that form as a result of the reaction of the nonionic hydrophilic monomer with the crosslinking agent. For example, these bonds form as a result of the reaction of the hydroxyl groups in the hydroxyalkyl acrylate with the N-(isobutoxymethyl)- acrylamide. In addition, amide-amide crosslinks also form as a result of the reaction between two N-(isobutoxymethyl)acrylamide monomers. For example, the amide-ester crosslink can have the formula:

$$-C(=O)O-R-NH-C(=O),$$

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably $-CH_2-CH_2-CH_2-O-CH_2-$. The amide-amide crosslink can have the formula:

$$-C(=O)NH-R-NH-C(=O),$$

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably $-CH_2-O-CH_2-$.

The coating solution contains the anionic polymer prepared as above and, optionally, a polysaccharide, preferably a dextran. The anionic polymer and the polysaccharide can be present in the coating solution in the ratio of from about 100:1 to about 1:100, preferably from about 10:1 to about 1:10, and more preferably from about 5:1 to about 1:5.

The coating solution contains the anionic polymer and, optionally dextran, in an amount of from about 0.01% to about

for example, the membrane preferably has a water flow rate above 5 mL/min/cm^2, and preferably above 10 mL/min/cm^2, e.g., from about 20 mL/min/cm^2 to about 160 mL/min/cm^2, and preferably from about 25 mL/min/cm^2 to about 100 mL/min/cm^2 at 24 inch Hg. The membrane is robust and can withstand high treatment fluid flow rates. Thus, the membrane can be subjected to flow rates up to 10 cm/min, for example, from about 1 cm/min to 10 cm/min at 10 psi (68.9 kPa). The membrane has an open water bubble point of below about 70 psi (482 kPa), e.g., from about 2.5 psi (9.39 kPa) to about 70 psi (482 kPa), and preferably from about 5 psi (34.47 kPa) to about 50 psi (344.7 kPa).

The membrane of the present invention has a high charge density. The charge density of the membrane can be measured by methods known to those of ordinary skill in the art. For example, the charge density can be measured by the membrane's ability to bind a positively charged dye. Illustratively, the membrane has a Methylene Blue dye binding capacity of at least about 10 mL, e.g., from about 10 mL to about 1000 mL, and preferably from about 100 mL to about 800 mL, when tested with a 10 ppm dye solution in water. Methylene Blue is a positively charged dye. The dye binding capacity is measured by filtering under a 24 inch Hg negative pressure, a 10 ppm by weight solution, pH 6.6, of Methylene Blue dye in a membrane disc of 25 mm diameter, and monitoring the volume of the filtrate until a trace of the dye begins to appear in the filtrate.

The membrane of the present invention has a high specific protein binding capacity. The membrane has a lysozyme specific binding capacity of above 10 mg/mL, e.g., from about 10 mg/mL to about 130 mg/mL and preferably from about 25 mg/mL to about 120 mg/mL. The specific binding capacity can be determined by the following illustrative method. A fluid containing a lysozyme protein in 10 mM MES buffer, pH 5.5, is filtered by passing through a membrane at 1 cm/min and the concentration of the protein in the filtrate is measured as a function of time. The concentration of the protein can be

determined spectrophotometrically, e.g., by measuring the

stack can be eluted under a gradient - 7 ml from 10 mM MES buffer at a pH of 5.5 to 1M NaCl-10 mM MES buff r at a pH of 5.5. The flow rate can be 4 ml/min. Cytochrome C elutes first, followed by lysozyme.

The following examples further illustrate the present invention but should not be construed in any way limiting the scope of the invention.

EXAMPLE 1

This Example illustrates a method of preparing a polymer composition for preparing an embodiment of the negatively charged membrane of the present invention.

2-Acrylamido-2-methyl-1-propanesulfonic acid, N-(isobutoxymethyl)acrylamide, and hydroxypropyl methacrylate were combined in a weight ratio of 8.0:2.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 12% by weight. Ammonium persulfate was used as the initiator at 0.3% by weight of the solution. The polymerization was carried out for a period of about 10-15 hours at ambient temperature (20-25°C). The resulting solution had a viscosity of 166 cps (0.166 Pa.s).

EXAMPLE 2

This Example illustrates a method for preparing an embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

A coating solution was prepared by mixing the polymerization solution described in Example 1 and a water solution of dextran, molecular weight 148 K, so that the resulting solution contains polymer and dextran in the weight ratio of 3:1.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 µm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI

first 10 minutes of the treatment confirmed that the membrane did not leak. The nearly vertical slope indicates that the m mbrane was capable of providing high resolution.

EXAMPLE 4

This Example illustrates a method for preparing another embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

2-Acrylamidoglycolic acid, 2-carboxyethyl acrylate, N-(isobutoxymethyl)acrylamide, N-(hydroxymethyl)-acrylamide, and hydroxypropyl acrylate were combined in a weight ratio of 5.0:5.0:3.0:1.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 16% by weight. Ammonium persulfate was used as the initiator at 0.4% by weight of the solution. The polymerization was carried out for a period of about 16-24 hours at ambient temperature. The resulting solution had a viscosity of 116 cps (0.116 Pa.s). A coating solution was prepared by mixing the polymerization solution and a water solution of dextran, molecular weight 148 K, so that the resulting solution contained 4% polymer and 1.33% dextran by weight.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 µm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI water for 1 hour. The resulting membrane was dried in an oven to obtain another embodiment of the present invention.

The membrane obtained above was tested with a solution containing lysozyme. The solution was contained 213.6 µg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 3. The membrane had a lysozyme binding capacity of 45 mg/ml. The relatively flat curve obtained during the first 10 minutes of the treatment confirmed that the membrane

WHAT IS CLAIMED IS:

1. A negatively charged microporous membrane comprising a porous substrate and a crosslinked coating, wherein the crosslinked coating is prepared from a polymer comprising an unsaturated monomer having a negatively charged group, a hydrophilic non-ionic unsaturated monomer, and at least one or more N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide.

2. The negatively charged microporous membrane of claim 1, wherein the hydrophilic non-ionic unsaturated monomer is an acrylic monomer.

3. The negatively charged microporous membrane of claim 1 or 2, wherein the N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide includes an alkyl group of 4 carbon atoms or less.

4. The negatively charged microporous membrane of any of claims 1-3, wherein the crosslinked coating includes a hydroxyl-rich material.

5. The negatively charged microporous membrane of claim 4, wherein the hydroxyl-rich material is a polysaccharide.

6. The negatively charged microporous membrane of any of claims 1-5, wherein said negatively charged group is a sulfonic or carboxylic acid.

7. The negatively charged microporous membrane of claim 2, wherein said acrylic monomer is an acrylate or acrylamide.

8. The negatively charged microporous membrane of claim 7, wherein said acrylic monomer is an acrylamide.

9. The negatively charged microporous membrane of claim 8, wherein said acrylamide is an alkylacrylamide.

10. The negatively charged microporous membrane of claim 9, wherein said acrylamide has a sulfonic acid group.

11. The negatively charged microporous membrane of claim 10, wherein said acrylamide is acrylamido-N-alkylsulfonic acid.

12. The negatively charged microporous membrane of claim 9, wherein said alkylacrylamide has a carboxylic acid group.

13. The negatively charged microporous membrane of claim 12, wherein said polymer includes a further acrylic monomer having a carboxylic acid group.

14. The negatively charged microporous membrane of claim 13, wherein said further acrylic monomer is an acrylate.

15. The negatively charged microporous membrane of claim 14, wherein said acrylate is β-carboxyethyl acrylate.

16. The negatively charged microporous membrane of claim 4, wherein said acrylic monomer is a hydroxyacrylic monomer.

17. The negatively charged microporous membrane of claim 16, wherein said hydroxyacrylic monomer is a hydroxyacrylamide or an hydroxyacrylate.

18. The negatively charged microporous membrane of any of claims 1-17, wherein said polymer includes an N-(alkoxymethyl)acrylamide.

19. The negatively charged microporous membrane of claim 5, wherein said polysaccharide is dextran.

20. The negatively charged microporous membrane of claim 1, wherein the polymer comprising an unsaturated monomer having a negatively

charged group, a hydrophilic non-ionic unsaturated monomer, and at least one or more N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide- is prepared by employing a free radical initiator.

21. The negatively charged microporous membrane of any of claims 1-20 having a dynamic protein binding capacity of about 25 mg/ml lysozyme or more.

22. A negatively charged microporous membrane comprising a porous substrate and a crosslinked coating comprising negatively charged groups and amide-amide and amide-ester crosslinks.

23. The negatively charged microporous membrane of any of claims 1-22, wherein said porous substrate comprises a substrate polymer.

24. The negatively charged microporous membrane of claim 23, wherein said substrate polymer is selected from the group consisting of polyaromatics, polysulfones, polyolefins, polystyrenes, polyamides, polyimides, cellulose acetates, cellulose nitrates, polycarbonates, polyesters, and fluoropolymers.

25. The negatively charged microporous membrane of claim 24, wherein said substrate polymer is a polysulfone.

26. The negatively charged microporous membrane of any of claims 1-25, wherein said porous substrate is hydrophilic.

27. A process for preparing a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having negatively charged groups, the process comprising:

(a) providing a porous substrate;

(b) contacting said substrate with a polymer comprising an unsaturated monomer having a negatively charged group, a hydrophilic non-ionic unsaturated monomer, and at least one or more of a N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide;

(c) curing the substrate obtained in (b) to obtain the negatively charged microporous membrane; and

(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

28. A process for preparing a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having negatively charged groups, the process comprising:

(a) providing a porous substrate;

(b) contacting said substrate with a polysaccharide and a polymer comprising an unsaturated monomer having a negatively charged group and an N-(hydroxymethyl)- or N-(alkoxymethyl)-acrylamide;

(c) curing the substrate obtained in (b) to obtain the negatively charged microporous membrane; and

(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

29. The process of claim 27 or 28, wherein said negatively charged group is a sulfonic or carboxylic acid.

30. The process of any of claims 27-29, wherein said unsaturated monomer having a negatively charged group is an acrylic monomer having a sulfonic or carboxylic acid group.

31. The process of claim 30, wherein said acrylic monomer having a sulfonic or carboxylic acid group is an acrylate or an acrylamide.

32. The process of claim 27, wherein the substrate is contacted in (b) with said polymer and a hydroxyl-rich material.

33. The process of any of claims 27-32, wherein said porous substrate comprises a substrate polymer.

34. The negatively charged microporous membrane prepared by the process of any of claims 27-33.

35. A device comprising the negatively charged microporous membrane of any of claims 1-26 and 34.

36. A process for separating positively charged material from a fluid, said process comprising placing said fluid in contact with the negatively charged microporous membrane of any of claims 1-26 and 34 so as to adsorb the positively charged material to said membrane.

37. The process of claim 36, wherein said positively charged material is a biomolecule.

38. A process for transferring biomolecules from an electrophoresis gel comprising contacting said electrophoresis gel with a membrane of any of claims 1-26 and 34 and transferring the biomolecules to the membrane.

39. The process of claim 38, wherein said biomolecule is selected from the group consisting of proteins, polypeptides, amino acids, and nucleic acids, and combinations thereof.

40. The process of claim 38 or 39, further including recovering the positively charged material adsorbed on the membrane.

NEGATIVELY CHARGED MEMBRANE

CROSS-REFERENCE TO A RELATED APPLICATION

This application claims priority from U.S. Provisional Patent Application No. 60/121,668, filed on February 25, 1999, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention generally relates to negatively charged membranes, and in particular to negatively charged membranes comprising a porous substrate and a crosslinked coating. The membranes find use in the treatment of fluids containing positively charged species such as proteins, e.g., in ion-exchange chromatography.

BACKGROUND OF THE INVENTION

Negatively charged ion-exchange membranes have been proposed for the separation and/or purification of biomolecules such as proteins, amino acids, and nucleic acids. For the ion exchange membrane to perform effectively in the above applications, the membrane should satisfy several important parameters. For example, the membrane should exhibit high rates of fluid flow. The membrane should have high dynamic binding capacity for biomolecules, and should be capable of selectively binding the biomolecules, which have different surface charges. The membrane should, therefore, have low non-specific binding, e.g., resulting from hydrophobic interactions. The membrane should withstand high treatment fluid velocities. The preparation of the membrane should not involve chemistries and processes that are cumbersome to practice. Some of the cation exchange membranes known heretofore suffer from the failure to satisfy one or more of the parameters set forth above.

Accordingly, there exists a need for a cation exchange membrane that exhibits high rates of fluid flow. There further exists a need for a cation exchange membrane that has

high dynamic binding capacity and selectivity for biomolecules. There further exists a need for a membrane that has low non-specific binding or low binding that results from hydrophobic interactions. There further exists a need for a membrane that can withstand high fluid flow velocities. There further exists a need for a membrane that involves preparation chemistries and/or processes that are not cumbersome to practice.

These advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the breakthrough curve for lysozyme obtained on an embodiment membrane of the present invention. The x-axis represents the filtration time, and the y-axis represents the absorbance of the filtrate at 280 nm and is indicative of the concentration of the protein. See Example 2 for additional details.

Fig. 2 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present invention. The x-axis and y-axis are as described in Fig. 1. See Example 3 for additional details.

Fig. 3 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present invention. The x-axis and y-axis are as described in Fig. 1. See Example 4 for additional details.

BRIEF SUMMARY OF THE INVENTION

Many of the foregoing needs have been fulfilled by the present invention which provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having negatively charged groups. In a preferred embodiment, the membrane can be prepared from a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-

(hydroxyalkyl)- and/or N-(alkoxyalkyl)- acrylamides, and a hydrophilic unsaturated monomer.

The present invention further provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating prepared from a hydroxyl-rich material such as a polysaccharide and a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-(hydroxymethyl)- and/or N-(alkoxymethyl)- acrylamides, and an initiator.

The present invention further provides a negatively charged microporous membrane having a protein binding capacity of about 25 mg/ml lysozyme or more comprising a porous substrate and a crosslinked coating that provides a fixed negative charge. The present invention further provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating comprising a polymer having anionic groups and amide-amide and amide-ester crosslinks.

The membranes of the present invention are advantageously free of covalent bonds or grafts with the substrate.

The present invention further provides a process for preparing an embodiment of the membrane comprising coating a porous substrate with a polymerized composition comprising an anionic group and curing the membrane. The membrane can be optionally washed or leached to remove extractable residue therein.

The present invention further provides devices, e.g., filter devices, chromatographic devices, macromolecular transfer devices, and membrane modules comprising the membranes of the present invention. The present invention further provides a process for separating a positively charged material such as positively charged atoms, molecules, and particulates, and, preferably, biomolecules, from a fluid, the process comprising placing the fluid in contact with the negatively charged microporous membrane so as to adsorb the positively charged material to the membrane. The present invention further provides a process for treating a fluid

containing positively charged materials comprising contacting the fluid with a membrane of the present invention and selectively releasing the positively charged materials. The present invention further provides a process for transferring macromolecules from a device or element such as an electrophoresis gel comprising contacting the gel with the membrane of the present invention and transferring the biomolecules to the membrane.

While the invention has been described and disclosed below in connection with certain preferred embodiments and procedures, it is not intended to limit the invention to those specific embodiments. Rather it is intended to cover all such alternative embodiments and modifications as fall within the spirit and scope of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides embodiments of negatively charged membranes having high charge density, high water flow rates, high dynamic protein binding capacity, and low non-specific protein binding capacity. The membranes of the present invention find use in cation exchange chromatography and in the separation and/or purification of charged species, especially biomolecules such as proteins.

The present invention provides, in some embodiments, a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having anionic groups. The crosslinked coating can be prepared from a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamides, a hydrophilic unsaturated monomer. The composition can further include an initiator. In preferred embodiments, the N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide is one wherein the alkyl moiety has 4 or less carbon atoms, and more preferably the alkyl moiety is methyl.

In certain embodiments, the membrane comprises a porous substrate and a crosslinked coating prepared from a

polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-(hydroxymethyl)- and/or N-(alkoxymethyl)- acrylamides, a hydroxyl-rich material such as a polysaccharide, and optionally an initiator. The present invention further provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating comprising a polymer having anionic groups and amide-amide and amide-ester crosslinks.

The membrane of the present invention contains fixed anionic groups. The anionic group can be any negatively charged group - sulfonic, carboxylic, phosphonic, and the like, preferably sulfonic or carboxylic acid groups. The coating composition comprises an unsaturated monomer having an anionic group. Any suitable unsaturated monomer - vinyl, vinyl aromatic, acrylic, or other monomer can be used.

The unsaturated monomer preferably is an acrylic monomer. The acrylic monomer can be an acrylate or an acrylamide. The acrylic monomer is preferably an acrylamide. The term "acrylamide" herein refers to unsubstituted as well as substituted monomers having a -C=C-(C=O)-N- moiety. The nitrogen and the C=C carbon atoms can be attached to hydrogen or other nonpolar substituents. An example of such substituents is alkyl. Thus, the substituted acrylamide can be alkylacrylamide. The term "alkyl" herein refers to an alkyl group having from 1 to about 10 carbon atoms, preferably from 1 to about 6 carbon atoms, and more preferably from 1 to about 3 carbon atoms. An example of an acrylamide monomer having a sulfonic acid group is acrylamido-N-alkylsulfonic acid, preferably 2-acrylamido-2-methyl-1-propanesulfonic acid. Preferred examples of acrylic monomers having a carboxylic acid group are 3-acrylamido-3-methylbutanoic acid (AMBA), 2-acrylamidoglycollic acid, and β-carboxyethyl acrylate.

In certain embodiments, the coating composition comprises a hydrophilic unsaturated monomer, e.g., a nonionic hydrophilic unsaturated monomer. Any suitable hydrophilic unsaturated monomer can be used, preferably an acrylic

monomer. The monomer contains one or more polar groups that contribute hydrophilicity. Examples of such groups include hydroxy, alkoxy, hydroxyalkyl, and amido. Preferred hydrophilic groups are hydroxyl and hydroxyalkyl. Thus, preferred hydrophilic acrylic monomers are hydroxyacrylic and hydroxyalkylacrylic. The acrylic monomer can be an acrylate ester or an acrylamide. An example of a preferred hydroxyalkyl acrylate monomer is hydroxypropyl methacrylate.

The coating composition comprises a crosslinking agent. Any suitable crosslinking agent known to those of ordinary skill in the art can be used. Preferred crosslinking agents include N-(alkoxymethyl)acrylamide and N-(hydroxymethyl)acrylamide. N-(isobutoxymethyl)acrylamide is further preferred.

The coating composition preferably comprises an initiator. Any suitable initiator - free radical initiator, photoinitiator, and the like, can be used. A free radical initiator is preferred. An example of a suitable free radical initiator is a persulfate such as ammonium persulfate.

Without being bound to any particular theory, it is believed that the use of the three monomers in certain embodiments contributes to increased spatial separation of charges. Thus, it is believed that the distance between the anionic groups is increased. This increased distance disfavors association of the anionic groups. Accordingly, inter- and/or intra- chain association of anionic groups is reduced compared to a system wherein only an anionic monomer and a crosslinking monomer are employed, particularly in a two monomer system wherein a hydrophilic or hydroxyl-rich material such as a polysaccharide is not employed. The reduced association makes the negatively charged groups available for interaction with positively charged molecules in the treated fluid. This results, for example, in enhanced dynamic protein binding capacity.

The membrane according to some embodiments is made from a coating composition that includes a hydroxyl-rich material, which may be a small molecule or a polymer having a plurality

of hydroxyl groups, e.g., two, three, four or more hydroxyl groups per molecule. Examples of hydroxyl-rich materials include polysaccharides and polyvinyl alcohol, preferably polysaccharides. Without being bound to any particular mechanism, it is believed that the hydroxyl groups of the hydroxyl-rich material involve in hydrogen bonding with the fluid. The saccharide ring repeat units exert steric effects. Operation of one or both of these mechanisms results in increased charge separation among the anionic groups. The increased charge separation is believed to reduce anion association and facilitate interaction between the anionic sites and the positively charged species in the treated fluid.

Any suitable polysaccharide can be used, preferably a water soluble polysaccharide. An example of a preferred polysaccharide is dextran. The molecular weight of the dextran is below about 40,000,000, e.g., from about 10,000 to about 2,000,000, preferably from about 10,000 to about 500,000, and more preferably from about 10,000 to about 300,000. Particular examples of suitable molecular weights include 110,000 and 148,000.

The coating composition of certain embodiments can be prepared by combining and polymerizing the acrylic monomer having an anionic group, the nonionic hydrophilic monomer, a crosslinking agent, and the initiator. In some embodiments, the coating solution is prepared by combining and polymerizing the acrylic monomer having an anionic group, the polysaccharide, the crosslinking agent, and the initiator.

The polymerization can be carried out in a solvent, preferably in water or water/methanol solution. The polymerization is preferably stopped prior to the formation of a gel or excessive crosslinking. The viscosity of the polymerization solution can be monitored to control the degree of polymerization. The polymerization is carried out for any suitable length of time, e.g., for about 4 hours or more. According to certain embodiments, the polymerization is carried out for a period of from about 4 hours to about 5 hours. According to certain other embodiments, the

polymerization is carried out for a period of from about 16 hours to about 24 hours. The viscosity of the solution is typically below about 2000 cps, e.g., from about 50 cps to about 500 cps, preferably from about 50 cps to about 500 cps, and more preferably from about 100 cps to about 500 cps. According to certain embodiments, the viscosity is from about 100 cps to about 250 cps.

The polymerization solution can contain the anionic acrylic monomer (A), the crosslinking agent (B), and the non-ionic hydrophilic monomer (C) in a suitable ratio. The percentage of each monomer (A, B, or C) can be from about 0.1 to 30% by weight, preferably from about 0.1 to 20% by weight.

It is believed that the crosslinked coating comprises amide-ester crosslinks that form as a result of the reaction of the nonionic hydrophilic monomer with the crosslinking agent. For example, these bonds form as a result of the reaction of the hydroxyl groups in the hydroxyalkyl acrylate with the N-(isobutoxymethyl)- acrylamide. In addition, amide-amide crosslinks also form as a result of the reaction between two N-(isobutoxymethyl)acrylamide monomers. For example, the amide-ester crosslink can have the formula:

-C(=O)O-R-NH-C(=O),

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably $-CH_2-CH_2-CH_2-O-CH_2-$. The amide-amide crosslink can have the formula:

-C(=O)NH-R-NH-C(=O),

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably $-CH_2-O-CH_2-$.

The coating solution contains the anionic polymer prepared as above and, optionally, a polysaccharide, preferably a dextran. The anionic polymer and the polysaccharide can be present in the coating solution in the ratio of from about 100:1 to about 1:100, preferably from about 10:1 to about 1:10, and more preferably from about 5:1 to about 1:5.

The coating solution contains the anionic polymer and, optionally dextran, in an amount of from about 0.01% to about

15% by weight, preferably from about 0.1% to about 10% by weight, and more preferably from about 0.5% to about 5% by weight of the coating solution. For example, the coating solution can contain 4.5% by weight of polymer and 1.5% by weight of dextran.

The pH of the coating solution can be adjusted suitably. For example, the pH of the coating solution containing a carboxylated polymer can be adjusted to about 3.0 to about 4.0 and preferably about 3.75. The pH of the coating can be adjusted by the addition of an acid or base. An example of a suitable base is 2N NaOH aqueous solution.

The coating solution is coated on a porous substrate, preferably a hydrophilic substrate. The hydrophilic porous substrate can be made of any suitable material; preferably, the substrate comprises a polymer. Examples of suitable polymers include polyaromatics, polysulfones, polyolefins, polystyrenes, polycarbonates, polyamides, polyimides, fluoropolymers, cellulosic polymers such as cellulose acetates and cellulose nitrates, and PEEK. Aromatic polysulfones are preferred. Examples of aromatic polysulfones include polyethersulfone, bisphenol A polysulfone, and polyphenylsulfone. Polyethersulfone is particularly preferred. The porous substrate can have any suitable pore size, for example, a pore size of below about 10 μm, e.g., from about 0.01 μm to about 10 μm, preferably from about 0.1 μm to about 5 μm, and more preferably from about 0.2 μm to about 5 μm. The porous substrate can be asymmetric or, in a preferred embodiment, symmetric.

The porous substrate can be prepared by methods known to those of ordinary skill in the art. For example, the porous substrate can be prepared by a phase inversion process. Thus, a casting solution containing the polymer, a solvent, a pore former, a wetting agent, and optionally a small quantity of a non-solvent is prepared by combining and mixing the ingredients, preferably at an elevated temperature. The resulting solution is filtered to remove any impurities. The casting solution is cast or extruded in the form of a sheet or

hollow fiber. The resulting sheet or fiber is allowed to set or gel as a phase inverted membrane. The set membrane is then leached to remove the solvent and other soluble ingredients.

The porous substrate can be coated with the coating solution by methods known to those of ordinary skill in the art, for example, by dip coating, spray coating, meniscus coating, and the like. Dip coating, for example, can be carried out as follows. The substrate is immersed in the solution for a given period of time sufficient to insure complete or substantially complete coating of the pore walls. The immersion time can be from about 1 second to 1.0 minute, preferably from about 0.1 minutes to about 0.5 minutes, and more preferably from about 1/6 minute to about 1/3 minute. Following the immersion, the excess coating solution on the substrate is removed by allowing it to drain under gravity or by the use of a squeegee or air knife. The resulting coated substrate is cured to effect the curing or crosslinking of the coating composition.

Thus, the membrane can be cured below 150°C, e.g., at a temperature of from about 60°C to about 130°C, and preferably at a temperature of from about 80°C to about 130°C, for a suitable period of time, which can range from about 5 minutes to about 120 minutes and preferably from about 5 minutes to about 60 minutes. According to certain embodiments, the membrane is cured at a temperature of from about 120°C to about 125°C for a period of from about 20 minutes to about 30 minutes.

The resulting membrane can be washed to leach out any extractable in the membrane. Certain embodiments of the membrane, particularly a membrane having carboxyl functionality, are washed or leached in a basic solution, preferably at a pH of from about 8 to about 12. The leaching liquid can be prepared by adding a base such as sodium hydroxide, sodium carbonate, or sodium bicarbonate. The base can be added as a solid or a solution. Particular examples of pH's of the leaching liquid are about 11.9, about 11.4, and

about 8.1. These pH's can be achieved by the use of, e.g., a 2N NaOH solution, sodium carbonate, or sodium bicarbonate.

Illustratively, a carboxylated membrane can be washed or leached at a temperature of from about 37°C to about 93°C or higher and preferably from about 54°C to about 73°C or higher. A sulfonic acid containing membrane can be washed or leached at a temperature of from about 54°C to about 93°C or higher. Embodiments of the membrane also can be leached in hot deionized water, e.g., deionized water held above 73°F. The washing or leaching can be carried out for a suitable length of time, for example, for about 20 to about 30 minutes or more. According to certain embodiments of the membrane, the washing or leaching can be carried out for about 1 hour or more. The resulting membrane is then dried in air or in an oven to remove the water.

The present invention provides a process for preparing a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having pendant anionic group. An embodiment of the process comprises:

(a) providing a porous substrate;

(b) contacting the substrate with a hydroxyl-rich material and a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-(hydroxyalkyl)- and/or N-(alkoxyalkyl)- acrylamides, a hydrophilic unsaturated monomer, and optionally an initiator;

(c) curing the substrate obtained in (b) to obtain the negatively charged membrane; and

(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

The present invention further provides a negatively charged membrane comprising a porous substrate and a crosslinked coating. An embodiment of the process comprises:

(a) providing a porous substrate;

(b) contacting the substrate with a polysaccharide and a polymerized composition comprising an unsaturated monomer having an anionic group, an N-(hydroxymethyl)- and/or N-(alkoxymethyl)- acrylamides, and an initiator;

(c) curing the substrate obtained in (b) to obtain the negatively charged membrane; and

(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

The present invention further provides, in an embodiment, a negatively charged microporous membrane comprising a porous support and a crosslinked coating wherein the crosslinked coating is prepared from a polymerized composition comprising an unsaturated monomer having an anionic group, an N-(hydroxymethyl)- or N-(alkoxymethyl)-acrylamide, a nonionic hydrophilic acrylic monomer, and an initiator.

The present invention further provides, in another embodiment, a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating prepared from a polysaccharide and a polymerized composition comprising an unsaturated monomer having an anionic group, an N-(hydroxymethyl)- or N-(alkoxymethyl)- acrylamide, and an initiator.

The present invention, in a further embodiment, provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating prepared from a composition comprising an acrylic monomer having an anionic group, an N-(hydroxymethyl)- or N-(alkoxymethyl)- acrylamide, a nonionic hydrophilic acrylic monomer, and an initiator.

The present invention, in another embodiment, provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating prepared from a polysaccharide and a polymerized composition comprising an acrylic monomer having an anionic group, an N-(hydroxymethyl)- or N-(alkoxymethyl)- acrylamide, and an initiator.

The present invention further provides a device e.g., a filter device, chromatography device, macromolecular transfer device, flow distributor arrangement, and/or a membrane module comprising one or more negatively charged membranes of the present invention. The device can be in any suitable form. For example, the device can include a filter element comprising the negatively charged membrane in a substantially

planar or pleated form. In an embodiment, the element can have a hollow generally cylindrical form. If desired, the device can include the filter element in combination with upstream and/or downstream support or drainage layers. The device can include a plurality of membranes, e.g., to provide a multilayered filter element, or stacked to provide a membrane module, such as a membrane module for use in membrane chromatography. Filter cartridges can be constructed by including a housing and endcaps to provide fluid seal as well as at least one inlet and at least one outlet. The devices can be constructed to operate in crossflow or tangential flow mode as well as dead-end mode. Accordingly, the fluid to be treated can be passed, for example, tangentially to the membrane surface, or passed perpendicular to the membrane surface.

For embodiments of the membrane which are in the form of a tube or fiber, or bundles of tubes or fibers, the membrane can be configured as modules, e.g., after potting their ends with an adhesive. For a description of illustrative chromatographic devices, porous medium modules, and flow distributor arrangements, see U.S. Provisional Patent Application Nos. 60/121,667 and 60/121,701, both filed on February 25, 1999; U.S. Provisional Patent Application Nos. 60/168,738 and 60/168,750, both filed on December 6, 1999; and International Applications filed on February 25, 2000 and entitled "Positively Charged Membrane" by Xiaosong Wu, Chung-Jen Hou, Jayesh Dharia, Peter Konstantin, and Yujing Yang; "Chromatography Devices and Flow Distributor Arrangements Used in Chromatography Devices" by Mark Hurwitz, Thomas Sorensen, John Stempel, and Thomas Fendya; and "Chromatography Devices, Porous Medium Modules Used in Chromatography Devices and Methods for Making Porous Medium Modules" by Mark Hurwitz, Thomas Fendya, and Gary Bush. See also UK Patent Application GB 2 275 626 A.

The membrane of the present invention has one or more advantageous properties, including high water permeability dynamic protein binding capacity, and charge density. Thus,

for example, the membrane preferably has a water flow rate above 5 mL/min/cm^2, and preferably above 10 mL/min/cm^2, e.g., from about 20 mL/min/cm^2 to about 160 mL/min/cm^2, and preferably from about 25 mL/min/cm^2 to about 100 mL/min/cm^2 at 24 inch Hg. The membrane is robust and can withstand high treatment fluid flow rates. Thus, the membrane can be subjected to flow rates up to 10 cm/min, for example, from about 1 cm/min to 10 cm/min at 10 psi. The membrane has an open water bubble point of below about 70 psi, e.g., from about 2.5 psi to about 70 psi, and preferably from about 5 psi to about 50 psi.

The membrane of the present invention has a high charge density. The charge density of the membrane can be measured by methods known to those of ordinary skill in the art. For example, the charge density can be measured by the membrane's ability to bind a positively charged dye. Illustratively, the membrane has a Methylene Blue dye binding capacity of at least about 10 mL, e.g., from about 10 mL to about 1000 mL, and preferably from about 100 mL to about 800 mL, when tested with a 10 ppm dye solution in water. Methylene Blue is a positively charged dye. The dye binding capacity is measured by filtering under a 24 inch Hg negative pressure, a 10 ppm by weight solution, pH 6.6, of Methylene Blue dye in a membrane disc of 25 mm diameter, and monitoring the volume of the filtrate until a trace of the dye begins to appear in the filtrate.

The membrane of the present invention has a high specific protein binding capacity. The membrane has a lysozyme specific binding capacity of above 10 mg/mL, e.g., from about 10 mg/mL to about 130 mg/mL and preferably from about 25 mg/mL to about 120 mg/mL. The specific binding capacity can be determined by the following illustrative method. A fluid containing a lysozyme protein in 10 mM MES buffer, pH 5.5, is filtered by passing through a membrane at 1 cm/min and the concentration of the protein in the filtrate is measured as a function of time. The concentration of the protein can be determined spectrophotometrically, e.g., by measuring the

absorbance of the protein at 280 nm. A breakthrough curve such as the one shown in Fig. 1 can then be constructed with the x-axis depicting the time of the filtration experiment and the y-axis depicting the protein concentration in the filtrate. The membrane has high specific protein binding capacity and low non-specific or hydrophobic binding. The slope of the breakthrough curve obtained on the membrane is vertical or substantially vertical. This characteristic offers improved resolution and separation of proteins. The membrane also has high dynamic protein binding capacity.

An advantage of the membrane of the present invention is that proteins do not leak prior to breakthrough. Another advantage of the present invention is that the components of the membrane are carefully chosen so that the membrane is free or substantially free of grafts or covalent links between the coating and the substrate. The preparation of negatively charged membranes of the present invention involves a chemistry and procedure that is relatively simple and easy to practice.

The properties of the membranes of the present invention make them attractive for use in the detection, separation, and/or purification of biomolecules such as proteins, amino acids, nucleic acids, and viruses. Examples of nucleic acids include modified or unmodified, synthetic or natural RNA and DNA.

The membranes of the present invention find use in various applications such as filtration of fluids containing positively charged atoms, molecules, and particulates, and macromolecular transfer from electrophoresis gels such as the transfer of nucleic acids and proteins from electrophoresis gels to an immobilizing matrix. The membrane can find use in the separation or purification of components present in biological fluids. Thus, for example, the membrane can find use in the purification of human albumins from the serum, in the therapeutic fractionation of blood, and separation of the components in genetically engineered cell cultures or fermentation broths. The membrane can be used in the

purification of, for example, viral vaccines and gene therapy vectors such as adeno-associated viruses.

Accordingly, the present invention provides a process for treating a fluid containing biomolecules, the process comprising placing the fluid in contact with the negatively charged membrane. The positively charged materials adsorbed on the membrane can be recovered by eluting with a suitable solvent eluant. The present invention further provides a process for selectively adsorbing one or more biomolecules from fluid containing a mixture of biomolecules comprising contacting the fluid with the membrane under conditions favorable to the adsorption of selected biomolecules. The present invention further provides a process for selectively releasing one or more biomolecules from a membrane of the present invention comprising contacting the membrane having adsorbed biomolecules with an eluant under conditions favorable to the release of the selected biomolecules. The present invention further provides a process for macromolecular transfer from an electrophoresis gel comprising contacting a membrane of the present invention with the electrophoresis gel, and transferring the macromolecules from the gel to the membrane.

The negatively charged membrane of the present invention is particularly suitable for treating fluids containing biomolecules that have a positive surface charge for the given pH of the fluid. For example, lysozyme has an isoelectric point of 11.25, and it can be purified by using the negatively charged membrane of the present invention from a low salinity, for example 10mM MES, fluid that is pH 5.5. Proteins with different surface charges may also be separated using the membrane of the present invention, for example separating lysozyme from Cytochrome C.

Thus, a mixture of lysozyme and Cytochrome C can be separated as follows. 80 µl of a fluid containing 3 mg/ml lysozyme and 1 Cytochrome C can be placed on a chromatographic column or stack of 5 layers of a 25 mm diameter negatively charged membrane of the present invention. The column or

stack can be eluted under a gradient - 7 ml from 10 mM MES buffer at a pH of 5.5 to 1M NaCl-10 mM MES buffer at a pH of 5.5. The flow rate can be 4 ml/min. Cytochrome C elutes first, followed by lysozyme.

The following examples further illustrate the present invention but should not be construed in any way limiting the scope of the invention.

EXAMPLE 1

This Example illustrates a method of preparing a polymer composition for preparing an embodiment of the negatively charged membrane of the present invention.

2-Acrylamido-2-methyl-1-propanesulfonic acid, N-(isobutoxymethyl)acrylamide, and hydroxypropyl methacrylate were combined in a weight ratio of 8.0:2.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 12% by weight. Ammonium persulfate was used as the initiator at 0.3% by weight of the solution. The polymerization was carried out for a period of about 10-15 hours at ambient temperature (20-25°C). The resulting solution had a viscosity of 166 cps.

EXAMPLE 2

This Example illustrates a method for preparing an embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

A coating solution was prepared by mixing the polymerization solution described in Example 1 and a water solution of dextran, molecular weight 148 K, so that the resulting solution contains polymer and dextran in the weight ratio of 3:1.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 μm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI

water for 1 hour. The resulting membrane was dried in an oven to obtain an embodiment of the present invention.

The membrane obtained above was tested for treatment of a solution containing lysozyme. The solution was contained 206.4 µg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 1. The membrane had a lysozyme binding capacity of 97 mg/ml. The relatively flat curve obtained during the first 10 minutes of the treatment confirmed that the membrane did not leak. The nearly vertical slope indicates that the membrane was capable of providing high resolution.

EXAMPLE 3

This Example illustrates a method for preparing an embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

A coating solution was prepared by mixing the polymerization solution described in Example 1 and a water solution of dextran, molecular weight 148 K, so that the resulting solution contains polymer and dextran in the weight ratio of 4:1.

A hydrophilic microporous cellulose nitrate substrate having a pore size of about 0.8 µm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI water for 1 hour. The resulting membrane was dried in an oven to obtain an embodiment of the present invention.

The membrane obtained above was tested with a solution containing lysozyme. The solution was contained 201.3 µg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 2. The membrane had a lysozyme binding capacity of 77 mg/ml. The relatively flat curve obtained during the

first 10 minutes of the treatment confirmed that the membrane did not leak. The nearly vertical slope indicates that the membrane was capable of providing high resolution.

EXAMPLE 4

This Example illustrates a method for preparing another embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

2-Acrylamidoglycolic acid, 2-carboxyethyl acrylate, N-(isobutoxymethyl)acrylamide, N-(hydroxymethyl)-acrylamide, and hydroxypropyl acrylate were combined in a weight ratio of 5.0:5.0:3.0:1.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 16% by weight. Ammonium persulfate was used as the initiator at 0.4% by weight of the solution. The polymerization was carried out for a period of about 16-24 hours at ambient temperature. The resulting solution had a viscosity of 116 cps. A coating solution was prepared by mixing the polymerization solution and a water solution of dextran, molecular weight 148 K, so that the resulting solution contained 4% polymer and 1.33% dextran by weight.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 μm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI water for 1 hour. The resulting membrane was dried in an oven to obtain another embodiment of the present invention.

The membrane obtained above was tested with a solution containing lysozyme. The solution was contained 213.6 μg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 3. The membrane had a lysozyme binding capacity of 45 mg/ml. The relatively flat curve obtained during the first 10 minutes of the treatment confirmed that the membrane

did not leak. The nearly vertical slope indicates that the membrane was capable of providing high resolution.

All references cited herein, including patents, patent applications, and publications, are incorporated by reference in their entireties.

While this invention has been described with an emphasis upon several embodiments, it will be obvious to those of ordinary skill in the art that variations of the embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. A negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having pendant anionic groups.

2. The negatively charged microporous membrane of claim 1, wherein the crosslinked coating is prepared from a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide, and a hydrophilic unsaturated monomer.

3. The negatively charged microporous membrane of claim 2, wherein the hydrophilic unsaturated monomer is nonionic.

4. The negatively charged microporous membrane of claim 3, wherein the hydrophilic unsaturated monomer is an acrylic monomer.

5. The negatively charged microporous membrane of claim 2, wherein the N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide includes an alkyl group of 4 carbon atoms or less.

6. The negatively charged microporous membrane of claim 1, wherein the crosslinked coating includes a hydroxyl-rich material.

7. The negatively charged microporous membrane of claim 6, wherein the hydroxyl-rich material is a polysaccharide.

8. The negatively charged microporous membrane of claim 6 or 7, wherein the coating further includes a polymerized composition comprising an unsaturated monomer having an anionic group and an N-(hydroxyalkyl)- or N-(alkoxyalkyl)-acrylamide.

9. The negatively charged microporous membrane of any of claims 1-8, wherein said anionic group is a sulfonic or carboxylic acid.

10. The negatively charged microporous membrane of claim 2 or 8, wherein the coating is prepared from composition that further includes an initiator.

11. The negatively charged microporous membrane of claim 3, wherein said unsaturated monomer is an acrylic monomer having a sulfonic or carboxylic acid group.

12. The negatively charged microporous membrane of claims 11, wherein said acrylic monomer is an acrylate or acrylamide.

13. The negatively charged microporous membrane of claim 12, wherein said acrylic monomer is an acrylamide.

14. The negatively charged microporous membrane of claim 13, wherein said acrylamide is an alkylacrylamide.

15. The negatively charged microporous membrane of claim 13, wherein said acrylamide has a sulfonic acid group.

16. The negatively charged microporous membrane of claim 15, wherein said acrylamide is acrylamido-N-alkylsulfonic acid.

17. The negatively charged microporous membrane of claim 13, wherein said monomer is an acrylamide having a carboxylic acid group.

18. The negatively charged microporous membrane of claim 17, wherein said composition includes a further acrylic monomer having a carboxylic acid group.

19. The negatively charged microporous membrane of claim 18, wherein said further acrylic monomer is an acrylate.

20. The negatively charged microporous membrane of claim 19, wherein said acrylate is β-carboxyethyl acrylate.

21. The negatively charged microporous membrane of claim 4, wherein said acrylic monomer is a hydroxyacrylic monomer.

22. The negatively charged microporous membrane of claim 21, wherein said hydroxyacrylic monomer is a hydroxyacrylamide or an hydroxyacrylate.

23. The negatively charged microporous membrane of any of claims 2-5, wherein said composition includes an N-(alkoxymethyl)acrylamide.

24. The negatively charged microporous membrane of claim 10, wherein said initiator is a free radical initiator.

25. The negatively charged microporous membrane of claim 2 or 6, wherein said polysaccharide is dextran.

26. The negatively charged microporous membrane of any of claims 2-5, wherein said composition further includes a polysaccharide.

27. The negatively charged microporous membrane of claim 26, wherein said polysaccharide is dextran.

28. A negatively charged microporous membrane having a dynamic protein binding capacity of about 25 mg/ml lysozyme or more comprising a porous substrate and a crosslinked coating that provides a fixed negative charge to the membrane.

29. A negatively charged microporous membrane comprising a porous substrate and a crosslinked coating comprising anionic groups and amide-amide and amide-ester crosslinks.

30. The negatively charged microporous membrane of any of claims 1-29, wherein said porous substrate comprises a substrate polymer.

31. The negatively charged microporous membrane of claim 30, wherein said substrate polymer is selected from the group consisting of polyaromatics, polysulfones, polyolefins, polystyrenes, polyamides, polyimides, cellulose acetates, cellulose nitrates, polycarbonates, polyesters, and fluoropolymers.

32. The negatively charged microporous membrane of claim 31, wherein said substrate polymer is a polysulfone.

33. The negatively charged microporous membrane of any of claims 1-33, wherein said substrate is hydrophilic.

34. A device comprising the negatively charged microporous membrane of any of claims 1-33.

35. A process for preparing a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having anionic groups, the process comprising:

(a) providing a porous substrate;

(b) contacting said substrate with a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more of a N-(hydroxyalkyl)- or N-(alkoxyalkyl)- acrylamide, a hydrophilic unsaturated monomer, and an initiator;

(c) curing the substrate obtained in (b) to obtain the negatively charged membrane; and

(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

36. A process for preparing a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having anionic groups, the process comprising:

(a) providing a porous substrate;

(b) contacting said substrate with a polysaccharide and a polymerized composition comprising an unsaturated monomer having an anionic group, an N-(hydroxymethyl)- or N-(alkoxymethyl)- acrylamide, and an initiator;

(c) curing the substrate obtained in (b) to obtain the negatively charged membrane; and

(d) optionally, extracting the membrane obtained in (c) to remove extractable residue therein.

37. The process of claim 35 or 36, wherein said anionic group is a sulfonic or carboxylic acid.

38. The process of claim 37, wherein said unsaturated monomer is an acrylic monomer having a sulfonic or carboxylic acid group.

39. The process of claim 38, wherein said acrylic monomer having a sulfonic or carboxylic acid group is an acrylate or an acrylamide.

40. The process of claim 35 or 36, wherein said coating composition further includes a hydroxyl-rich material.

41. The process of any of claims 35-40, wherein said porous substrate comprises a substrate polymer.

42. The negatively charged membrane prepared by the process of any of claims 35-41.

43. A process for separating positively charged material from a fluid, said process comprising placing said fluid in contact with the negatively charged microporous membrane of any of claims 1-44 and 62 so as to adsorb the positively charged material to said membrane.

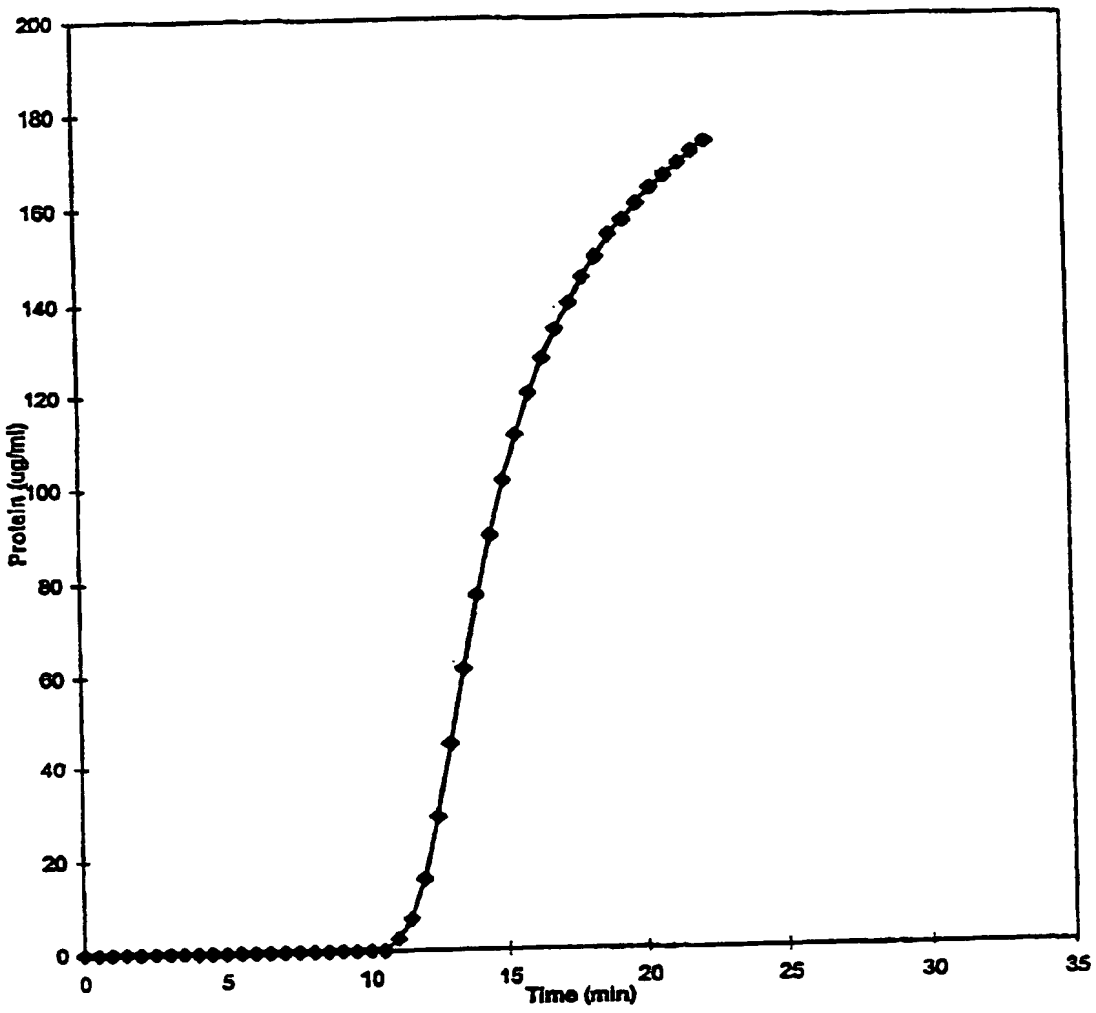
44. The process of claim 43, wherein said material is a biomolecule.

45. A process for transferring biomolecules from an electrophoresis gel comprising contacting said gel with a membrane of any of claims 1-44 and 42 and transferring the biomolecules to the membrane.

46. The process of claim 45, wherein said biomolecule is selected from the group consisting of proteins, polypeptides, amino acids, and nucleic acids, and combinations thereof.

47. The process of claim 43 or 44, further including recovering the positively charged material adsorbed on the membrane.

Figure 1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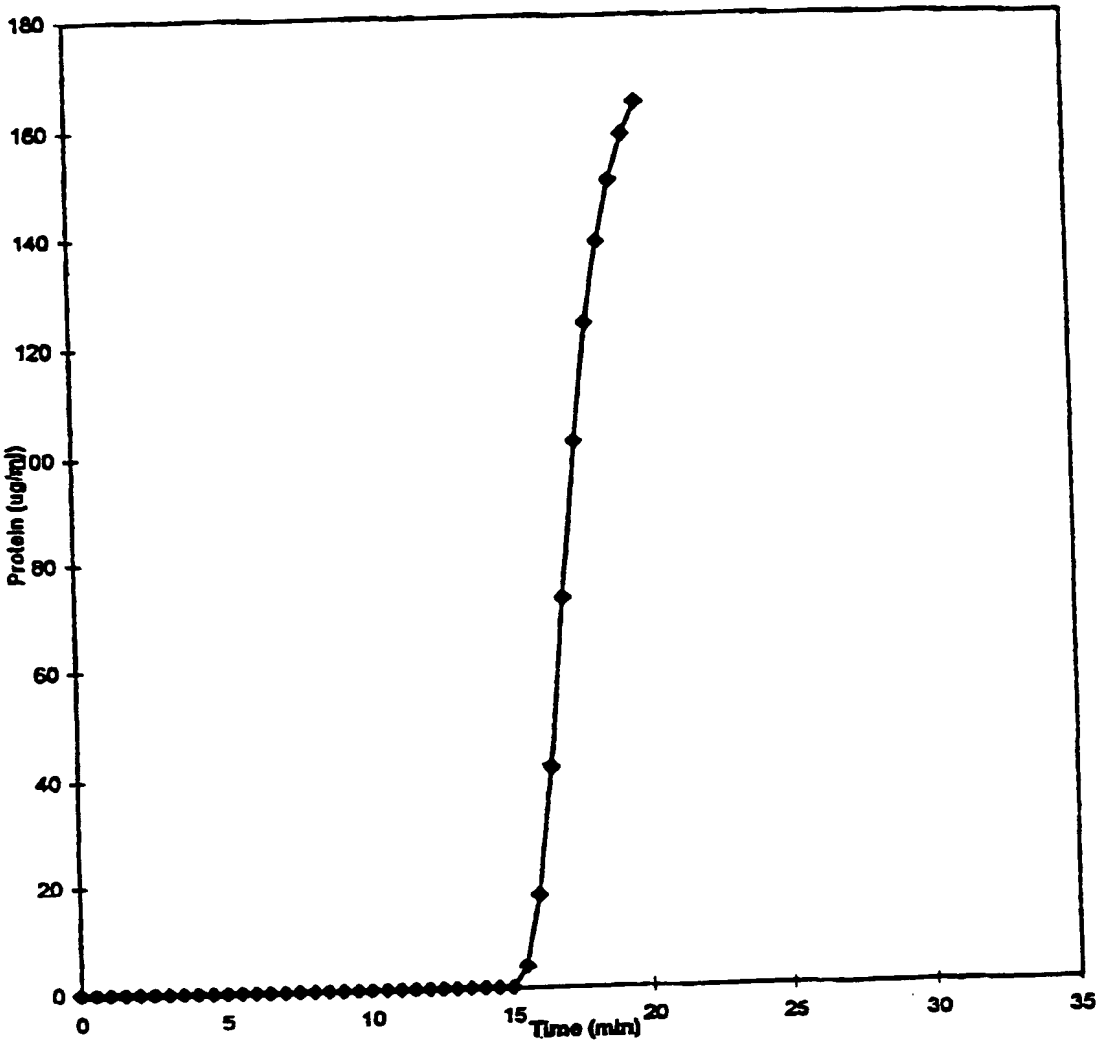
Time (min)

Figure 2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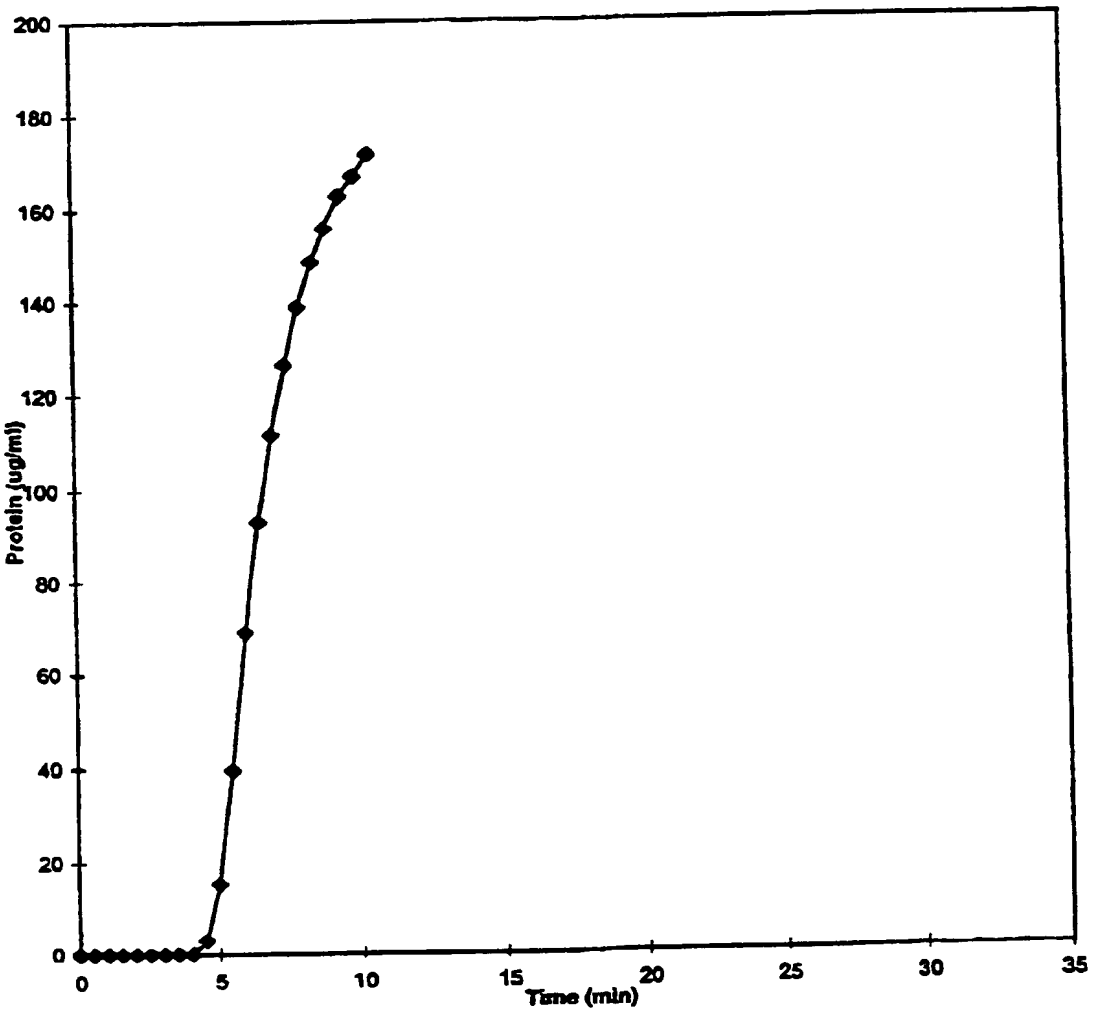
Time (min)

Figure 3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Protein (ug/ml)
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35
Time (min)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 00/04745

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01D67/00 B01D61/00 B01J39/20 B01J47/12 B01J20/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01D B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

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11 July 2000	19/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hilgenga, K

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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
B01D 69/10, 69/14, 67/00, 71/28, 71/78, 71/80, 71/82

A1

(11) International Publication Number: **WO 98/17377**

(43) International Publication Date: 30 April 1998 (30.04.98)

(21) International Application Number: PCT/CA97/00770

(22) International Filing Date: 17 October 1997 (17.10.97)

(30) Priority Data:
08/733,792 18 October 1996 (18.10.96) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MICROPOROUS MEMBRANES AND USES THEREOF

(57) Abstract

Charged membranes comprise a porous substrate and a cross-linked polyelectrolyte or hydrogel located in the pores of the substrate and are useful in a variety of membrane separation processes, including pressure driven membrane separation, diffusion dialysis, Donnan dialysis, electrodialysis, electrochemical synthesis and pervaporation.

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TITLE OF INVENTION

MICROPOROUS MEMBRANES AND USES THEREOF

FIELD OF INVENTION

The present invention relates to certain novel membranes and the novel uses of certain membranes.

FIELD OF INVENTION

This application is a continuation-in-part of US Patent Application No. 08/733,792 filed October 18, 1996.

BACKGROUND OF THE INVENTION

Membranes are used, for instance, in separation processes as selective barriers that allow certain chemical species to pass, i.e., the permeate, while retaining other chemical species, i.e., the retentate. Membranes are used in many applications, for example as biosensors, heparinized surfaces, facilitated transport membranes utilizing crown ethers and other carriers, targeted drug delivery systems including membrane-bound antigens, catalyst-containing membranes, treated surfaces, sharpened resolution chromatographic packing materials, narrow band optical absorbers, and in various water treatments which involve removal of a solute or contaminant, for example, dialysis, electrodialysis, microfiltration, ultrafiltration, reverse osmosis, nanofiltration and in electrolysis and in fuel cells and batteries.

There are a large number of supports or substrates for membranes. Specific physical and chemical characteristics to be considered when selecting a substrate include: porosity, surface area, permeability, solvent resistance, chemical stability, hydrophilicity, flexibility and mechanical integrity. Other characteristics may be important in certain applications.

In Mika et al., J. Membr. Sci., 108 (1995) pp 37 to 56, there is described a procedure for modifying microporous polypropylene and polyethylene membranes wherein 4-vinylpyridine is *in situ* polymerized into the pores of the membrane.

SUMMARY OF INVENTION

We have found that, by cross-linking the membranes described by Mika et al. with a suitable cross-linking agent, such as divinylbenzene (DVB), there are provided charged membranes comprising porous microfiltration substrate membranes whose pores have located therein a cross-linked polyelectrolyte or hydrogel anchored to the substrate polymer, which exhibit novel effects in a variety of membrane applications.

In particular, the membranes exhibit significant ion rejection properties, enabling water softening to be effected, particularly at ultra-low pressure, such as the pressure of tap water, by removing multivalent ions, such as calcium and magnesium, in preference to monovalent ions, such as sodium.

The membranes further exhibit electrochemical separator properties which make them suitable for a wide variety of applications, including electrodialysis, battery separators, fuel cell separators and electrochemical synthesis.

In addition, the membrane may be used for Donnan dialysis, diffusion dialysis and pervaporation.

Accordingly, in one aspect of the present invention, there is provided an improvement in a membrane separation process selected from the group consisting of pressure driven membrane separation, diffusion dialysis, Donnan dialysis, electrodialysis, electrochemical synthesis and pervaporation, the improvement which comprises employing a charged membrane comprising a porous substrate and a cross-linked polyelectrolyte or hydrogel located in the pores of the

substrate. Certain of the charged membranes are novel, as set forth in the claims herein and described below.

The polyelectrolyte or hydrogel may be found in the pores of the substrate by *in situ* polymerization of a monomer or a mixture of monomers with a cross-linking agent, the monomer or at least one of the monomer mixture being selected from those monomers which contain a functional group that provides an ion-exchange site and those which contain a group which is susceptible to a chemical reaction by which such functional groups are subsequently introduced to the *in situ*-formed polymer.

Alternatively, the polyelectrolyte or hydrogel may be formed in the pores of the substrate by, first, *in situ* polymerization of a monomer or a mixture of monomers, the monomer or at least one of the monomers of the monomer mixture being selected from those monomers which contain a functional group that provides an ion-exchange site and those which contain a group which is susceptible to a chemical reaction by which such functional groups are subsequently introduced to the *in situ*-formed polymer, and, subsequently, cross-linking the *in situ*-formed polymer.

The properties of the cross-linked polyelectrolyte or hydrogel located in the pores of the substrate, by covalent bonding to or cross-linked around structural elements of the porous substrate may be modified for specific applications by selection of the appropriate degree of cross-linking.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1, comprising graphs A and B, contains a graphical representation of the effects of temperature on pervaporation of water/ethanol mixtures, as detailed in Example 7 below.

GENERAL DESCRIPTION OF INVENTION

The porous microfiltration substrate which is modified to provide the charged membranes used herein

may comprise a porous substrate formed of polymeric material, such as polypropylene or polyethylene, into the pores of which may be *in situ* polymerized and cross-linked polyelectrolytes or hydrogels anchored to the substrate polymer by either covalent bonding to or cross-linked around the structural elements of the porous substrate.

For porous substrates, the pore diameters may vary widely but preferably range from about 0.01 to about 20 microns, more preferably from about 0.1 to about 5 microns and particularly from about 0.2 to about 1.0 microns. Pore diameters for microporous substrate are measured by the bubble-point method according to ASTM F-316.

The porosity or pore volume of a polymeric porous substrate used herein is preferably from about 25 to about 95%, more preferably from about 45 to about 85% and particularly from about 60 to about 80%. Porosity can be derived from the value of the bulk density of the porous substrate and the polymer density of substrate polymer according to ASTM D-792.

The thickness of substrate will depend on the intended use of the membrane product. For many uses, for example microfiltration, thicknesses ranging from about 1 to about 1000 microns, more preferably about 10 to about 240 microns and particularly about 20 to about 100 microns, would be suitable.

In situ polymerization of a suitable monomer to enable anchoring of polymeric molecules having ionizable groups may be effected by any convenient polymerization procedure, preferably by free-radical polymerization operation. Such free radical polymerization may include initiation of the polymerization by radiation initiation, thermal initiation or redox initiation. Typical initiators which may be used in the free radical polymerization include benzoin ethers and benzoyl

peroxide. The *in situ* polymerization may include graft polymerization.

Monomers which are suitable for such *in situ* polymerization include unsaturated derivatives containing a functional group that provides, or can be modified by a post-polymerization treatment to provide, an ion-exchange site to permit formation of a polyelectrolyte or hydrogel. The membrane which is formed may be anionic or cationic, depending on the unsaturated monomer which is *in situ* polymerized. Suitable examples include 4-vinylpyridine, acrylic acid, methacrylic acid, styrene, vinylbenzyl chloride and acrylamido-alkyl-sulfonic acid, such as 2-acrylamido-2-methyl-1-propane sulfonic acid. The polymers so formed in the pores are non-extractable therefrom and hence anchored therein.

The cross-linking of the *in-situ* polymerized molecule to control or modulate conformational flexibility of such molecules may be effected by adding the cross-linking monomer to the *in-situ* polymerized monomer, so that the *in-situ* polymerization and cross-linking occur simultaneously. Alternatively, the cross-linking may be effected as a separate operation following the initial *in-situ* polymerization. The cross-linking which is formed may be covalent or ionic in nature and may be effected by radiation cross-linking.

The simultaneous *in situ* polymerization and cross-linking is preferred since the yield of the *in-situ* polymerization in terms of increase over the base weight of the substrate, is significantly increased thereby.

The cross-linking agent may be any suitable unsaturated molecule capable of reacting to produce cross-links in the *in-situ* polymerized molecules. The cross-linking agent may be a molecule containing at least two unsaturated moieties to permit the formation

of cross-links. Examples of such monomers are divinylbenzene and divinylpyridine. Other examples of suitable cross-linking monomers are diacrylates, such as di(ethylene glycol) diacrylate, tetra(ethylene glycol) diacrylate or 1,6-hexanediol diacrylate.

The quantity of cross-linking monomer used depends on the membrane application and may vary up to about 30 wt% of the total weight of *in situ* polymerized monomer mixture. For water treatment under low pressure driven applications, the quantity of cross-linking monomer may run up to about 10%, preferably from about 0.25 to about 5 wt% of total weight *in situ* polymerized monomer mixture. For the electrodialysis, diffusion dialysis and Donnan dialysis applications, the quantity of cross-linking monomer may vary from about 0.25 to about 30 wt%, preferably from about 15 to about 25 wt% of total weight of *in situ* polymerized monomers.

The polyelectrolytes may be cross-linked after they have been formed *in situ* in the pores by a post-polymerization treatment. The cross-linking agent used in this type of post-polymerization cross-linking may be a molecule containing at least two or more functional groups capable of reacting with functional groups or other active sites on the *in situ* formed polymer to form covalent bonds or ionic bonds. Examples of molecules forming covalent bonds are dialkylating reagents, such as 1,3-dibromopropane, diacylating and triacylating reagents, such as isophthaloyl and trimesoyl chlorides, respectively. Examples of ionic cross-linking include complexes formed between multivalent transition metal ions and carboxylic acid groups.

The quantity of *in situ*-formed polymer depends on the membrane application and may vary from about 20 to about 400 wt% of the initial weight of the polymeric porous substrate. For water treatment under low pressure driven applications, the quantity of *in situ*-formed

polymer may vary from about 30 to about 200 wt%, preferably from about 45 to about 100 wt% of weight of the polymeric porous substrate. For electrodialysis, diffusion dialysis and Donnan dialysis applications, the quantity of *in situ*-formed polymer may vary from about 50 to about 250 wt%, preferably from about 150 to about 250 wt% of polymeric porous substrate.

The amine type nitrogen atoms of incorporated polymers may be quaternized for certain applications, such as by alkylation, for example, with dimethyl sulphate, as well as alkyl halides, including arylalkyl halides.

Particular combinations of monomers for production of the cross-linked polyelectrolyte or hydrogel which may be employed include:

- an *in-situ* formed copolymer of vinylpyridine and a monomer selected from divinyl benzene and divinylpyridine,
- an *in-situ* formed polyvinylpyridine which is subsequently cross-linked with an alkylating agent, such as 1,3-dibromo-propane,
- an *in-situ* copolymer of vinylbenzylchloride and divinylbenzene into which the ion-exchange functional groups are introduced by reaction with a tertiary amine,
- an *in-situ* formed copolymer of styrene and divinylbenzene into which the ion-exchange functional groups are introduced by sulfonation,
- an *in-situ* formed copolymer of acrylic acid or methacrylic acid and divinylbenzene,
- an *in-situ* formed copolymer of acrylic acid or methacrylic acid and a diacrylate.
- an *in-situ* formed copolymer of acrylic acid or methacrylic acid and tetra(ethyleneglycol) diacrylate.

- an *in-situ* formed copolymer of an 2-acrylamido 2-methyl-1-propane sulfonic acid and tetra(ethyleneglycol) diacrylate.

Microporous polypropylene or polyethylene membranes which have about 45 to about 100 wt% by weight of polymeric porous substrate of *in situ* polymerized vinylpyridine and which are cross-linked with about 0.25 to about 5 wt% by weight of the total monomers by divinylbenzene are particularly useful in pressure driven water treatment, i.e. reverse osmosis or nanofiltration, possessing the property to reject multivalent cations in preference to monovalent cations. By varying the degree of the amount of the *in situ*-formed polymer and the degree and properties of the cross-linking, the membrane may be modified to be specific for specific applications. For electrodialysis, diffusion dialysis and Donnan dialysis applications, microporous polypropylene or polyethylene membranes which have about 150 to about 250 wt% by weight of polymeric porous substrate of *in situ* polymerized vinylpyridine and which are cross-linked with about 15 to about 25 wt% by total weight of the monomers by divinyl-benzene are particularly useful.

Ion rejection and use of charged membranes:

The charged membranes, comprising a non-ionic, porous substrate having pores which are filled with a cross-linked polyelectrolyte bound to or around the structural elements of the substrate polymer, are capable of rejecting both inorganic and organic ions from water at pressures as low as 345 kPa (50 psig), a pressure which is within the range of tap water delivery pressure. Such preferential rejection is seen at even lower pressures down to 140 kPa (20 psig).

The rejection of salts containing monovalent cations, for example, Na^+, is substantially lower than rejection of salts with multivalent cations, for

example, Mg^{2+}, Ca^{2+}. Charged organic materials, such as organic acids and salts, also are rejected by the membranes, while relatively large non-ionic organic molecules, such as sucrose, have low rejections by the membranes. The ability of the membranes to function at such ultra-low pressures and their distinctive pattern of separations distinguishes the membranes from commercially available nanofiltration or reverse osmosis membranes, which function only effectively at higher pressures and generally exhibit high rejections of large non-ionic organic molecules.

Unlike commercial membranes, the pore-filled membranes provided herein exhibit quite a different dependence of the ratio of permeate flux with a salt solution as feed to permeate flux with pure water as feed on pressure. At low pressures, a 0% DVB cross-linked grafted material has a permeate to pure water flux which exceeds 1. This ratio decreases with increasing pressure due either changes in the membrane itself or concentration polarization. With a 1% cross-linking, the ratio at low pressure is reduced somewhat below 1 but is essentially pressure independent. With 4% cross-linking, the membrane starts to behave much more like a typical commercial thin-film composite membrane.

The ability of the membranes provided herein to effect ultra-low pressure ion-rejection has wide application of use in water treatment technology to soften water without removing most non-ionic organic matter from water. Such applications may range from domestic water softening operations to the removal of calcium from tap water supplied to air conditioning systems as well as to water softening applications generally.

Existing commercial membranes used for water softening are limited by an excessive and indiscriminate

rejection of all dissolved species and this is particularly true with thin-film composite membranes, commercial examples being low-pressure nanofiltration membranes available from FilmTec and Fluid Systems. Other nanofiltration membranes which have been developed specifically for removal of organic materials from water, generally humic acid derivatives, exhibit a low removal of ions, including calcium. The recommended operating pressures for commercially available low pressure nanofiltration membranes are higher than those found to be sufficient for the invented membranes.

Diffusion Dialysis

The technologies currently employed for treating waste acid streams generally involve neutralization and solid waste disposal. The costs of such a disposal routine are increasing rapidly and environmental concerns and the value of recovering of a variety of metal ions, for example, chromium, are strong incentives for treatment of these waste streams.

The charged membranes provided herein are useful in diffusion dialysis of solutions containing mineral acids and metal salts to separate the salts from the acids, with the acids being transported through the membranes at high rates while the salts are rejected by the membranes. The degree of cross-linking employed in the membranes used in diffusion dialysis is generally greater than for pressure driven processes. The permeability of the membranes to protons is not much affected by cross-linking, up to a certain level. However, water permeability and metal ion permeability are affected. The membranes are also suitable for separating acids from neutral organic compounds under diffusion dialysis conditions.

Diffusion dialysis with the charged membranes can be used for the recovery of acid and stabilization of electrolyte composition in a number of industrial

processes, such as in the almite process, in aluminum capacitor etching, purification and metal salt recovery in non-ferrous smelting and refining, stabilization of electrolytic etching solutions and treatment of spent pickling solutions in secondary processing of iron and steel, and in purification of industrial acids, such as sulfuric acid and hydrochloric acid.

Electrochemical and related processes and uses of charged membranes

Charged membranes are used in a wide variety of electrochemical applications including electrodialysis, electrolysis, fuel cells and battery separators. A key feature of membranes for these applications are high ion-exchange capacities, low water transport, low electrical resistance, and good selectivity in terms of the transport of ions of different charge type (cations versus anions).

The charged membranes provided herein are useful in the applications, such as electrodialysis, electrochemical processes, fuel cells and batteries. In particular, they have very high ion-exchange capacities, exceeding 4 milli-equivalents per gram, and very low electrical resistances. The measured resistances are independent of cross-linking degree at least for the range of about 1 up to about 5 wt%, thereby allowing control over water permeability by using more highly cross-linked polyelectrolytes within the pores. Such membranes constitute a further aspect of the invention.

Pervaporation

Pervaporation is a process in which a liquid feed solution is placed in contact with a membrane on the other side of which is a vapor phase. Generally, the vapor phase is held at a partial vacuum. Components in the liquid phase are transported through the membrane, evaporate on the vapor side of the membrane and are subsequently condensed for recovery. Selectivity in

separation of the components in the feed is achieved by the proper choice of membrane material. Pervaporation is widely used in the final dehydration of ethanol.

The membranes provided herein are useful in pervaporation processes showing very high overall fluxes and good separations. They can be used, for example, in the purification of ethanol/water streams.

EXAMPLES

In the specific Examples which follow, polypropylene (PP) or polyethylene (PE) microporous substrates were used which had an average pore diameter of about 0.2 μm, a thickness of about 50 μm and a porosity of about 65 to 70 volume percent. Such polypropylene substrates were made following the procedure described in U.S. Patent No. 4,726,989 (Marozinski) while the polyethylene substrates were made following the procedure described in U.S. Patent No. 4,539,265 (Shipman), the disclosures of such United States patents being incorporated herein by reference.

Example 1:

This Example illustrates the preparation of membranes.

The PP and PE substrates were subjected to *in situ* polymerization of 4-vinylpyridine (4VP) with varying amounts of divinylvenzene (DVB) to provide anion-exchange membranes. Divinylbenzene of technical grade containing 55% of a mixture of monomers, was purchased from Aldrich Chemical Company, St. Louis, MO and was initially purified by vacuum distillation. All reagents employed in the membrane preparations described herein were purchased from Aldrich Chemical Company.

A. Thermally-initiated *in situ* polymerization:

In thermally-initiated *in situ* polymerization from the vapor phase, the porous PP or PE substrate was coated with benzoyl peroxide (BPO) by immersing it in an acetone solution containing 1% BPO and 1% poly(vinyl

acetate) for 5 to 10 minutes and subsequent drying it in air. The coated substrate was suspended inside a glass reactor containing on its bottom 2 to 3 mL of a vinylpyridine/DVB mixture. After the pressure inside the reactor had been reduced below 10 mmHg, the reactor was heated to 80°C for half an hour to effect the polymerization.

B. Photo-initiated *in situ* polymerization:

In photo-initiated *in situ* polymerization from solution, the porous PP or PE substrate was wetted with vinylpyridine, DVB and 1 to 1.5% of benzoin ethyl ether as a photo-initiator. The wetted substrate was degassed in a freeze-thaw cycle and irradiated using light of wavelength 350 nm for 30 minutes.

In each such procedure, unbound homopolymer was removed from the membranes by extraction with boiling methanol until no further mass loss occurred.

C. Quaternization:

Quaternization of amine groups in the *in situ* formed cross-linked polymer was effected by immersing the membrane into a solution containing 5 to 10% by volume of dimethyl sulfate in methanol at room temperature for 16 to 24 hours followed by subsequent thorough wash of the membrane with methanol and, finally, with deionized water. In an alternative procedure, the membrane was immersed into a solution containing about 5 wt% of dimethylsulfate in N,N-dimethyl-formamide at room temperature for 30 to 60 minutes followed by subsequent thorough wash of the membrane with deionized water.

D. Cross-linking with 1,3-dibromopropane:

Quaternization and cross-linking of amine groups in the *in situ* formed cross-linked polymer was effected with 1,3-dibromopropane carried out using a solution that contained 0.05 mol of 1,3-dibromopropane per 1 mol of pyridine nitrogen in the membrane dissolved in 100 to

150 mL of methanol. The membrane was placed in the solution and heated under reflux for 70 hours.

E. Cross-linking with α,α'-dibromo-ρ-xylene:

Quaternization and cross-linking of amine groups in the *in situ*-formed polymer was also carried out with α,α'-dibromo-ρ-xylene using a solution that contained 0.5 g of α,α'-dibromo-ρ-xylene in 80 mL of methanol. The ratio of α,α'-dibromo-ρ-xylene to pyridine nitrogen in the membrane was 5 to 1. The membrane was placed in the solution and heated under reflux for 16 hours.

Example 2

This Example shows the water softening capability of the membranes prepared as described in Example 1, in comparison to known membranes, as described by Fu et al., Journal AWWA, 86, 55 to 72 (1994).

A. Commercially-available membranes:

Four commercially-available thin-film composite membranes were tested for their ability to reject organic and inorganic components. Table I provides the chemical and physical characteristics of the membranes while Table II provides the performance data.

TABLE I

Characteristics of thin-film composite (TFC)

nanofiltration membranes

Membrane	Material	Rated Operating Pressure kPa (psig)	Flux at Rated Pressure L/m^{2}h (gpd/sq ft)	Permeability L/m^{2}h kPa (gpd/sq ft psig)
NF70[1]	modified aromatic polyamide	483 (70)	37 (22)	0.118 (0.48)
TFCS[2]	modified aromatic polyamide	552 (80)	26 (15)	0.049 (0.20)
NTR7450[3]	sulfonated polyether sulfone	986 (143)	93 (55)	0.106 (0.43)
NTR7410[3]	sulfonated polyether sulfone	986 (143)	496 (292)	0.185 (0.75)

(1) FilmTec, Minneapolis, Minn.
(2) Fluid Systems, San Diego, CA.
(3) Nitto Denko from Hydranautics, San Diego, CA.

TABLE II

Rejection (%) of organics and inorganics by TFC nanofiltration membranes

Membrane	Color	TOC	Conductivity	Alkalinity	Calcium
NF70	>97.5	94	90	93	98.5
TFCS	>97.5	96	92	94	98.5
NTR7450	>97.5	93	30	32	35.0
NTR7410	97.0	86	10	5	N/A

B. Membranes of Example 1:

(a) A membrane prepared as described in Example 1 by the photo-initiated *in-situ* polymerization procedure was tested for its water softening ability on untreated tap water alone or in combination with organic materials at a flux of 2.52 L/m^2h at 345 kPa (50 psig). The membrane was a polypropylene base membrane *in situ* polymerized with 4-vinylpyridine containing 1.2% divinylbenzene. This membrane was subsequently quaternized by treatment with dimethyl sulphate as described in Example 1. The results obtained for individual runs of approximately 24 hours, which were reproducible over long term testing, are set forth in the following Table III:

TABLE III

COMPONENT	RUN 1[1]		RUN 2[2]		RUN 3[3]	
	Feed, ppm	Rejection, %	Feed, ppm	Rejection, %	Feed, ppm	Rejection %
Sodium	19.5	71.3	13.3	38.8	13.3	60.0
Magnesium	12.0	97.4	10.4	58.6	8.9	91.0
Calcium	49.8	92.6	41.9	57.1	36.4	82.1
Acetate			115.8	48.2		
Chloride			16.9	51.0		
Sulfate			UD[4]	99.5+		
Sucrose					558.4	12.9

Notes:
(1) Run 1 = Tap water
(2) Run 2 = Tap water + 100 ppm acetic acid
(3) Run 3 = Tap water + 550 ppm sucrose
(4) UD = undetectable due to precipitation of $CaSO_4$ at increased concentrations of Ca^{2+} in the feed.

As may be seen from these data, the charged membranes effected water softening since they remove calcium and other bivalent ions to a much larger extent than sodium ions. The results also show that the membranes are able to remove charged organics (acetate).

Operation at 50 psig permits the membranes to be driven directly from a municipal water supply, with no pretreatment and with no additional pressurization being required and at pressures significantly lower than the commercially-available membranes shown in Table 1 and 2.

(b) Two different membranes prepared as described in Example 1 by the thermally-initiated vapor phase *in situ* polymerization (Membrane A) and the photochemical *in situ* polymerization method (Membrane B) were tested for their water-softening ability on untreated tap water. Membrane A was a polypropylene membrane *in situ* polymerized with 4-vinylpyridine containing 1.1 wt% of divinylbenzene. This membrane was subsequently quaternized by treatment with dimethyl sulphate as described in Example 1. Membrane B was a polypropylene membrane *in situ* polymerized with 4-vinylpyridine containing 1.2 wt% divinylbenzene. This membrane was subsequently quaternized by treatment with dimethyl sulphate as described in Example 1 (same membrane as Example 2(B)(a)).

The results are set forth in the following Table IV:

TABLE IV

Ion	Feed ppm	Membrane A Rejection % 345 kPa (50 psi)	Membrane A Rejection % 140 kPa (20 psi)	Membrane B Rejection % 345 kPa (50 psi)
Sodium	23.3	45.9	12.7	63.6
Magnesium	22.4	82.1	61.6	90.8
Calcium	85.5	66.4	29.7	88.4
Chloride	44.8	68.5	31.1	77.0
Sulphate	13.4	89.6	56.0	>99.5
Flux (L/m^2h)		5.76	2.12	2.52

These results show that substantial water softening is achieved at conventional tap pressures and that a pressure as low as 20 psi still provided substantial water softening.

Example 3

This Example illustrates the flux and rejection of cations from tap water using membranes prepared as described in Example 1.

Several different membranes, prepared following both the thermally-initiated and photoinitiated *in situ* polymerization procedures of Example 1, were tested for their flux and the ability to reject cations from tap water under a pressure of 345 kPa (50 psi). The results obtained are summarized in the following Table V:

TABLE V

No.	Membrane Characteristics			Flux kg/m^{2}h	Rejection, %		
	Substrate	%DVB	Mass Gain, %		Na	Mg	Ca
Membranes prepared by photoinitiated polymerization							
1	PP	3.0	212.4	1.0-1.7[a]	51	68	56
2	PP	1.2	147.0	2.3	71	97	93
3	PP	0.5	60.5	4.1	40	65	59
4	PP	0.5	131.6	2.5	67	82	80
5	PP	0.3	124.7	4.1	47	60	60
6	PE	0.3	209.8	3.8	53	83	77
7	PE	0.00	67.5	11.5	6	8	8
Membranes prepared by thermally-initiated vapour-phase polymerization							
10	PP	1.0	350.9	5.5	62	85	80

Notes: [a] obtained with two samples prepared under identical conditions; measured under 1000 kPa and extrapolated to 345 kPa (50 psi)

As may be seen from the results set forth in the above Table V, membranes produced by photoinitiated polymerization exhibit several characteristics. By comparing experiments 3 and 4, it can be seen that the flux decreases with mass gain. Flux also decreases with increasing levels of cross-linking monomer(experiments 4 and 5). The separation level generally increases with increasing levels of cross-linking monomer. A trade-off exists among quantity of *in situ*-formed polymer, cross-linking, flux and separation. The polyethylene substrate produced membranes with higher fluxes than the polypropylene substrate for the same level of cross-linking and had a higher mass gain.

Example 4

This Example further illustrates the flux and rejection of cations from tap water using membranes prepared as described in Example 1 at a higher cross-linking and lower incorporation levels in comparison to Example 3.

A membrane was prepared generally following the photoinitiated *in situ* polymerization procedure of Example 1 to provide a polyethylene microporous membrane (PE) having a incorporation of 58% poly(4-vinyl-pyridine) cross-linked with 4% divinybenzene and quaternized.

The membrane was prepared by a photochemical grafting (anchoring) procedure using 2,2-dimethoxy-2-phenylacetophenone as initiator. The contacting solution in the photografting was vinylpyridine with 4% divinylbenzene as a cross-linker diluted with pyridine, with the ratio of vinylpyridine/divinylbenzene to pyridine was 80:20. The presence of the pyridine leads to an improved uniformity in incorporation. The membrane was quaternized by treatment with dimethyl sulfate in dimethylformamide, which is a better solvent for nucleophilic substitution reactions than methanol and

allows not only to reduce the reaction time to less than an hour but also makes the reaction less sensitive to impurities, such as moisture.

Tests were conducted using this membrane at 500 kPa (72.5 psig) and converted to a temperature of 25°C for flux and the ability to reject cations from tap water and to reject sucrose from aqueous solution thereof. The membrane was cleaned by treatment with aqueous HCl (0.01M) after tap water tests and after sucrose tests, which restored the properties of the membrane to their original values. The membrane was also tested at 345 kPa (50 psig) and 100 kPa (145 psig) on tap water. The results obtained at 500 kPa (72.5 psig) are outlined in the following Table VIA:

TABLE VIA

Feed	Flux ($kg/m^2/h$)	Separation Na^+ (%)	Separation Mg^{2+}	Separation Ca^{2+}	Separation Sucrose
De-ionized Water	41	-	-	-	-
54 ppm NaCl	48	62	-	-	-
109 ppm NaCl	52	43	-	-	-
Tap Water	46	26	36	36	-
60 ppm NaCl plus 547 ppm sucrose	41	49	-	-	12
De-ionized Water	43	-	-	-	-
126 ppm NaCl	43	41	-	-	-

The results obtained at 345 kPa (50 psig) and 100 kPa (14.5 psig) are set forth in the following Table VIB:

TABLE VIB

Pressure	Flux ($kg/m^2/h$)	Separation Na^+ (%)	Separation Mg^{2+}	Separation Ca^{2+} (%)
345 kPa (50 psig)	36	26	38	37
100 kPa (14.5 psig)	12	9	30	28

As may be seen from the data presented in Tables VIA and VIB, using a microporous polyethylene substrate, the flux of the membrane has been increased in comparison to the results shown in Table V in Example 3. This result has been achieved by increasing the degree of cross-linking coupled with a decrease in the amount of material contained within the pores.

As compared to the results in Table V, there is some loss in separation which may be restored by increasing the loading, at the expense of flux. While fouling of the membrane occurred during the course of the experiments, the membranes were restored to their initial performance values by a simple dilute acid wash.

As may be seen from Table VIA, the tested membrane gave a very low separation of sucrose, confirming the data shown in Table III. This result contrasts with the results obtained under the same conditions using a typical commercial nano-filtration membrane (Osmonics BQ01 membrane), as set forth in the following Table VII:

TABLE VII

Feed	Flux ($kg/m^2/h$)	Separation Na^+ (%)	Separation Mg^{2+}	Separation Ca^{2+}	Separation Sucrose
De-ionized Water	39	-	-	-	-
54 ppm NaCl	46	83	-	-	-
109 ppm NaCl	48	68	-	-	-
Tap Water	33	19	27	31	-
60 ppm NaCl plus 547 ppm sucrose	31	51	-	-	61
De-ionized Water	28	-	-	-	-
126 ppm NaCl	27	28	-	-	-

As may be seen from the data in Table VII, a steady decline in flux occurred during the experiments, which was not restored by the cleaning cycle. As also may be seen, this commercial membrane had a high separation of sucrose in contrast to the results in Table VI, although in other respects the results are comparable.

A further comparison was made under the same process conditions with a Hydranautics nanofiltration prototype membrane (7450) and the results are set forth in the following Table VIII:

TABLE VIII

Feed	Flux ($kg/m^2/h$)	Separation Na^+ (%)	Separation Mg^{2+}	Separation Ca^{2+}	Separation Sucrose
De-ionized Water	14	-	-	-	-
54 ppm NaCl	14	86	-	-	-
109 ppm NaCl	14	77	-	-	-
Tap Water	14	33	65	68	-
60 ppm NaCl plus 547 ppm sucrose	15	72	-	-	97
De-ionized Water	16	-	-	-	-
126 ppm NaCl	15	58	-	-	-

As may be seen from the data in Table VIII, this membrane exhibits higher separation than achieved in Table VIA but at a substantially lower flux. A very high sucrose separation is marked contrast to the results of Table VIA. In addition, which the flux remained constant throughout the experiments, there was a loss of separation of NaCl with time and cleaning cycles did not restore the separation.

As may be seen from the data presented in this Example, the membranes used in accordance with the invention exhibited much better long term stability than the commercial membranes, comparable or better separations and quite different behaviour with sucrose/salt mixtures.

Example 5

This Example illustrates the use of the membranes for diffusion dialysis.

A membrane prepared as described in Example 1 comprising a polypropylene substrate having poly(4-vinylpyridine) (P4VP) and 3.3% DVB copolymerized in the pores thereof, was tested for diffusion dialysis of hydrochloric acid and sodium chloride in comparison to a commercially-available diffusion dialysis membrane Selemion DSV or AMV.

The results appear in the following Table IX:

TABLE IX

Membrane	Concentr. of Acid mol/L	Concentr. of Salt mol/L	Permeability, U, mol/(m²h (mol/L))		U_{HCl}/U_{NaCl}
			HCl	NaCl	
Selemion DSV or AMV	0.1	0.05	1.1	0.025	44
Example 1	0.1	0.05	14.0	1.4	10

Selemion DSV is a commercially available diffusion dialysis membrane, one of the few on the market. As can clearly be seen from Table IX, the permeability for the membranes provided herein is nearly 1.4 orders of magnitude larger than that of the DSV membrane. The selectivity is poorer by a factor of 4 for the membrane provided herein.

Example 6

This Example illustrates the effect of changing the degree of cross linking introduced in the *in situ* polymerization as well as post-polymerization cross-linking with 1,3-dibromopropane on diffusion dialysis.

Membranes were prepared as in Example 1. The membrane listed as Membrane D in the following Table X was the same as Membrane C except for a post-polymerization treatment with 1,3-dibromopropane. Both membranes C and D had a polypropylene substrate with P4VP and 0.3 % DVB *in situ* copolymerized in the pores thereof. Membranes E and F had 1.1% and 2.2% DVB cross-linking.

The membranes C, D, E and F were tested for diffusion dialysis with hydrochloric acid and sodium chloride in a flow cell. The membranes C, D. E and F provided herein were compared with the commercially available Selemion AMV membrane. The results obtained are set forth in the following Table X:

TABLE X

Membrane	Concentr. of Acid mol/L	Concentr. of Salt mol/L	Permeability, U, mol/(m^2h (mol/L))		U_{HCl}/U_{NaCl}
			HCl	NaCl	
Selemion AMV	1.0	0.5	4.3	0.07	61
Membrane C	1.0	0.5	58	14	4
Membrane D	1.0	0.5	104	13	8
Membrane E	1.0	0.5	60	7	9
Membrane F	1.0	0.5	80	8	10

The data shown in Table X show that increased cross-linking (up to 2.2% of cross-linker) with DVB gives membranes with higher acid permeabilities with increased selectivity. The additional cross-linking with dibromopropane further improves the membrane performance.

Example 7

This Example illustrates the effect of the concentration of cross-linker on selectivity and water permeability in diffusion dialysis recovery of acid.

Membranes were prepared generally according to the procedure of Example 1 by absorbing a solution of 4-vinylpyridine and varying amount of divinylbenzene with 2,2-dimethoxy-2-phenylacetophenone as photoinitiator into the polypropylene substrate and irradiating at 350 nm for approximately one hour.

Diffisuion dialysis testing was performed using a stirred cell with a feed solution consisting of 1 M HCl, 0.5 M NaCl and 0.5 M $MgCl_2$ and a permeate cell initially containing deionized water.

The results obtained are set forth in the following Table XI:

TABLE XI

Membrane	%Graft Yield	% DVB X-Linker	$U(H^+)$[a]	$\frac{U(H^+)}{U(Na^+)}$[b]	$\frac{U(H^+)}{U(Mg^{2+})}$[b]	Water Transport[c]	IEC (meq/g)[d]	Thickness (μm)[e]	IE Conc. (eq/L)[f]
422	147	0.54	45	8.8	20	0.2	4.53	131	2.2
406	157	1.3	45	9.5	26	0.5	4.37	107	2.5
415	173	2.5	40	11	31	0.3	4.7	120	2.8
412	172	4.1	33	13	51	0.3	4.8	96	3.3
417	182	6.1	34	13	47	0.3	4.3	94	3.6
407	47	4.1	47	6.6	13	0.1	2.2	69	1.3
408	52	4.1	47	6.7	12	0.3	2.4	71	1.3
426	191	4.1	35	13	42	0.5	4.4	99	3.2
421	210	4.1	37	13	41	0.3	4.41	106	3.2
420	203	4.0	37	13	41		4.88	102	2.9
428	181	8.3	32	16	64	0.3	3.97	96	3.5
431	201	15.4	23	16	152	0.15	3.81	89	4.5
432	208	24.8	16	35	378	0.08	351	71	5.3
412c	172	85% DBP[g]	23	36	294	0.2		74	
412f	172	95% DBX[h]	16	75	554	0.2		102	
Sel. DSV			5.9	100	2500	0.02		116	

[A] U= permeability: mol/hr•m^2•M

[b] Errors± 5 - 10%

[c] mL/ hr•m^2•M

[d] IEC= Ion Exchange Capacity, determined as in: A, M. Mika et al, J. Memb. Sci. 108,1995, pp 37-56.

[e] Measured in 1 M HCl by pycnometry.

[F] IE Conc. = Ion Exchange Concentration as equivalents of determined nitrogen per litre of water in the membrane.

[g] DBP = 1,3-dibromopropane : percent additional crosslinking calculated from mass gain.

[H] DBX = α, α'-dibromo-*p*-xylene : percent additional crosslinking calculated from mass gain.

The results set forth in Table XI show that the membrane selectivity is enhanced and water permeability reduced by substantial increases in the degree of crosslinking. The membranes outperformed the commercial membrane, Selemion DSV.

Example 8

This Example provides the membrane electrical resistance of certain of the membranes provided herein.

The electrical properties of membranes prepared following the procedures of Example 1 were determined for various levels of cross-linking and compared with those of two commercial cation and anion exchange membranes, respectively Selemion CMV and AMV.

The results are contained in the following Table XII:

TABLE XII

Membrane Crosslinking %	Mass Gain % weight	Thickness μm	IEC meq/g	R/Ω (Cell)
4.5	195	90	3.01	0.02
3.4	186	117	3.16	0.03
2.2	251	126	3.26	0.03
1.1	170	109	3.18	0.04
Selemion CMV		150	ca 1.5	0.18
Selemion AMV		150	ca 1.5	0.36

As may be seen from the above Table XII, the electrical resistance of the membrane is very low. The resistance of the membrane is, within the error limits of the measurements, independent of the degree of cross-linking. As water permeability decreases with increased cross-linking, it appears that membranes optimized for electrodialysis will have relatively high cross-linker ratios, since water transport is unwanted in electrodialysis and many other electrochemical operations.

Transference numbers (t_+ and t_-) of the membrane having 4.5% DVB cross-linked therein are compared with the Selenion AMV in the following Table XIII:

TABLE XIII

	t_+	t_-
4.5% DVB Membrane	< 0.2	> 0.8
Selemion AMV	< 0.06	> 0.94

The high t_- and low t_+ values for the membrane containing 4.5% DVB implies that the membrane exchanges anions and rejects cations to a large degree, which is borne out by the water softening data contained in Example 2.

Example 9

The Example shows the use of the membranes for pervaporation.

Using a membrane prepared as described in Example 1 containing 4.5% DVB, the pervaporation properties were measured using an aqueous solution of ethanol containing 4% ethanol. The effect of temperature on separation factor (i.e. water selectivity) and flux were determined and plotted graphically. These data appear in Figure 1. As seen in graph A, the separation factor increased with temperature. As seen in graph B, the flux also increased with temperature.

The effect of ethanol concentration was also tested. The results obtained are shown in the following Table XIV:

TABLE XIV

Feed Solution	Temperature °C	Flux (kg/m^2h)	Separation Factor
4 wt% ethanol	50	0.3	4
85 wt% ethanol	50	2.4	11

Based on the results seen in Table XIV, it can be concluded that the membrane is water selective.

Example 10

This Example illustrates the preparation of cation-exchange membrane.

A. A first series of cation-exchange membranes was prepared using a polypropylene (PP) microporous substrate with a pore filler derived by photopolymerization of a 50 wt% methacrylic acid solution in water using benzophenone as a photoinitiator, and employing either divinylbenzene or tetra(ethyleneglycol) diacrylate as a cross-linking agent, following the procedure of Example 1.

A first membrane (BT10) comprised poly(methacrylic acid with 1% divinylbenzene and had an incorporation yield of 123%. This membrane was evaluated for the water-softening ability, as described in the following Example.

A second membrane (BT12) comprised poly(methacrylic acid) with 2% tetra(ethyleneglycol) diacrylate and had an incorporation yield of 120%. The measured ion-exchange capacity was 5.5 meq/g.

B. A second series of cation-exchange membranes were prepared from a PP microporous substrate having poly(2-acrylamido-2-methyl-1-propane sulphonic acid) anchored in the pores and lightly cross-linked with tetra(ethyleneglycol) diacrylate. The polymerizations were carried out in the pores of the substrate using 1 part of 2-acrylamido-2-methyl-1-propane dissolved in a mixture of water (1 part) and methanol (1 part), the diacrylate cross-linker and benzophenone as photoinitiator. Incorporation yields ranged from 150 to 400%. The performance of one of these membranes having 4% cross linking, in pressure-driven water treatment was examined, as outlined below.

Example 11

This Example illustrates the water softening capability of cation exchange membranes.

A. Membrane BT10, prepared as described in Example 10, was tested for the water softening ability on tap water at 354 kPa (50 psig) at a flux of 1.22 kg/m^2h. the rejection achieved was as follows:

Na^+	16%
Mg^{++}	61%
Ca^{++}	65%
Cl^-	5%
SO_4^-	42%

The separations which were achieved using the cation-exchange membrane based on poly(methacrylic acid) are comparable to those achieved using the anion-exchange membranes based on poly(4-vinylpyridine) at comparable fluxes.

B. Membrane BT16, prepared as described in Example 10, was tested for its water softening ability at 345 kPa (50 psig) in the treatment of tap water and in single salt separations at 483 kPa (70 psig). The rejection achieved on tap water (50 psig) at a flux rate of 1.9 kg/m^2h was as follows:

Na^+	14%
Mg^{++}	29%
Ca^{++}	31%
Cl^-	20%
SO_4^-	51%

The results obtained for single salt separations (70 psig) are set forth in the following Table XV:

TABLE XV

SALT (0.002M)	FLUX (KG/M²H. AT 70 PSIG)	REJECTION
NaCl	2.95	65
$CaCl_2$	2.7	19
$NaSO_4$	3.17	93

The fluxes achieved with these cation-exchange membranes were high and comparable to the poly(vinylpyridine) based membranes. The pattern of separations observed with the single salts in Table XV was that expected for a negatively-charged membrane.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides membranes having unique properties in a variety of applications. Modifications are possible within the scope of this invention.

CLAIMS

What we claim is:

1. In a membrane separation process selected from the group consisting of pressure driven membrane separation, diffusion dialysis, Donnan dialysis, electrodialysis, electrochemical synthesis, and pervaporation, the improvement which comprises employing a charged membrane comprising a porous substrate and a cross-linked polyelectrolyte or hydrogel located in the pores of the substrate.

2. The process of claim 1 wherein said membrane comprises a microporous substrate and about 20 to about 400 wt% weight of microporous substrate of a cross-linked in-situ polymerized polyelectrolyte or hydrogel located in the pores of the substrate and cross-linked by up to about 30 wt% of the polymerized monomers in the polyelectrolyte or hydrogel.

3. The process of claim 1 wherein said membrane separation process comprises a pressure-driven membrane separation to effect selective removal of multivalent cations from an aqueous medium containing monovalent cations and multivalent cations.

4. The process of claim 2 wherein, in said microporous membrane, the quantity of in-situ polymerized polyelectrolyte or hydrogel is about 30 to about 200 wt% and the amount of cross-linking monomer is up to about 10 wt% of the in-situ polymerized monomers.

5. The process of claim 4 wherein the quantity of in-situ polymerized polyelectrolyte or hydrogel is about 45 to about 100 wt% and the amount of cross-linking monomers is about 0.25 to about 5 wt%.

6. The process of claim 2 which is effected at a pressure of 70 psig (500 kPa) or less.

7. The process of claim 6 wherein said membrane comprises a microporous substrate in the pores of which is *in situ* polymerized from about 45 to about 100 wt% of

the substrate of vinylpyridine which is cross-linked with from about 0.25 to about 5 wt% of total monomers by divinylbenzene.

8. The process of claim 7 wherein amine groups in the polyelectrolyte are quaternized.

9. The process of claim 1 wherein said membrane separation process comprises electrodialysis, diffusion dialysis or Donnan dialysis and wherein, in said microporous membrane, the quantity of in-situ polymerized polyelectrolyte or hydrogel is about 50 to about 250 wt% and the amount of cross-linking monomers is from about 0.25 to about 30 wt% of the in-situ polymerized monomers.

10. The process of claim 9 wherein the quantity of in-situ polymerized polyelectrolyte or hydrogel is about 150 to about 250 wt% and the amount of cross-linking monomers is about 15 to about 25 wt%.

11. The process of claim 1 wherein said membrane separation process comprises electrodialysis, diffusion dialysis or Donnan Dialysis and wherein said microporous membrane comprises a microporous substrate having about 150 to about 250 wt% of the substrate of a cross-linked polyelectrolyte or hydrogel located in the pores of the substrate, said polyelectrolyte or hydrogel being polymerized 4-vinylpyridine which is cross-linked with about 15 to about 25 wt% of total polymerized monomers in said polyelectrolyte or hydrogel, by divinylbenzene and exhibiting a low electrical resistance and a low water permeability.

12. The process of claim 1 wherein the polyelectrolyte or hydrogel is formed in the pores of the substrate by *in situ* polymerization of a monomer or a mixture of monomers with a cross-linking agent, the monomer or at least one of the monomers of the monomer mixture being selected from those monomers which contain a functional group that provides an ion-exchange site and those which

contain a group which is susceptible to a reaction by which such functional groups are subsequently introduced to *in situ*-formed polymer.

13. The process of claim 1 wherein the polyelectrolyte or hydrogel is formed in the pores of substrate by, first, *in situ* polymerization of a monomer or a mixture of monomers, the monomer or at least one of the monomers of the monomer mixture being selected from those monomers which contain a functional group that provides an ion-exchange site and those which contain a group which is susceptible to a chemical reaction by which such functional groups are subsequently introduced to the *in situ*-formed polymer, and, subsequently, cross-linking *in situ*-formed polymer.

14. The process of claim 1 wherein the polyelectrolyte is a copolymer of vinylpyridine and a monomer selected from divinylbenzene and divinylpyridine.

15. The process of claim 13 wherein the polyelectrolyte is polyvinylpyridine cross-linked with a dialkylating reagent after *in situ* polymerization.

16. The process of claim 15 wherein said dialkylating agent is 1,3-dibromopropane or α,α'dibromo-ρ-xylene.

17. The process of claim 14 wherein the polyvinylpyridine is quaternized with an alkyl or aryl substituted alkyl halide or sulphate.

18. The process of claim 1 wherein the polyelectrolyte is selected from the group consisting of (1) copolymers of vinylbenzyl chloride and divinylbenzene and the ion-exchange functional groups are introduced by reaction with a tertiary amine; (2) copolymers of styrene and divinylbenzene and the ion-exchange functional groups are introduced by sulfonation; (3) copolymers of acrylic acid and divinylbenzene; (4) copolymers of methacrylic acid and divinylbenzene; (5) copolymers of acrylic acid and a diacrylate; or (6) copolymers of methacrylic acid and a diacrylate.

19. The process of claim 1 wherein the substrate is a microporous polyolefin substrate.

20. The process of claim 19 wherein the polyolefin is polypropylene or polyethylene.

21. The process of claim 1 wherein the properties of the bound polyelectrolyte or hydrogel are modified for a specific membrane and separation process by selection of the degree and type of cross-linking of the polyelectrolyte or hydrogel.

22. A charged membrane comprising a microporous polypropylene or polyethylene substrate and about 45 to about 100 wt% of a cross-linked polyelectrolyte or hydrogel located in the porous of the substrate, said cross-linked polyelectrolyte or hydrogel being polymerized 4-vinyl ridine which is cross-linked with from about 0.25 to about 5 wt% of total polymerized monomers in said polyelectrolyte by divinylbenzene.

23. The charged membrane of claim 22 wherein amine groups in said polyelectrolyte or hydrogel are quaternized by reaction with a quaternizing agent.

24. The charged membrane of claim 23 wherein said quaternizing agent is an alkyl or arylalkyl halide or a sulphate.

25. The charged membrane of claim 24 wherein said quaternizing agent is dimethyl sulphate.

26. A charged membrane comprising a microporous polypropylene or polyethylene substrate and a cross-linked polyelectrolyte or hydrogel located in the pores thereof which is further cross-linked by reaction with a cross-linking agent.

27. The charged membrane of claim 26 wherein said cross-linking agent is 1,3-dibromopropane or α,α'dibromo-p-xylene.

28. The charged membrane of claim 26 wherein said cross-linked polyelectrolyte or hydrogel is a copolymer of vinylpyridine and a monomer selected from

divinylbenzene and divinylpyridine.

29. The charged membrane of claim 28 wherein amine groups in said polyelectrolyte or hydrogel are quaternized.

30. The charged membrane of claim 29 wherein said amine groups are quaternized by reaction with dimethylsulphate.

31. A charged membrane comprising a microporous polypropylene or polyethylene substrate and about 150 to about 250 wt% of the substrate of a cross-linked polyelectrolyte or hydrogel located in the pores of the substrate, said polyelectrolyte or hydrogel being polymerized 4-vinylpyridine which is cross-linked with about 15 to about 25 wt% of total polymerized monomers in said polyelectrolyte or hydrogel, by divinylbenzene and exhibiting a low electrical resistance and a low water permeability.

32. The charged membrane of claim 31 wherein said cross-linked polyelectrolyte or hydrogen is a copolymer of vinylpyridine and a monomer selected from divinylbenzene and divinylpyridine.

33. The charged membrane of claim 31 wherein said polymerized 4-vinylpyridine is quaternized.

34. The charged membrane of claim 33 wherein said polymerized 4-vinylpyridine is quaternized with dimethylsulphate.

35. A charged membrane comprising a microporous substrate and about 20 to about 400 wt% weight of microporous substrate of a cross-linked in-situ polymerized polyelectrolyte or hydrogel located in the pores of the substrate and cross-linked by up to about 30 wt% of the polymerized monomers in the polyelectrolyte or hydrogel.

36. The charged membrane of claim 35 which is suitable for water treatment applications wherein the quantity of in-situ polymerized polyelectrolyte or hydrogel is about

30 to about 200 wt% and the amount of cross-linking monomer is up to about 10 wt% of the in-situ polymerized monomers.

37. The charged membrane of claim 36 wherein the quantity of in-situ polymerized polyelectrolyte or hydrogel is about 45 to about 100 wt% and the amount of cross-linking monomers is about 0.25 to about 5 wt%.

38. The charged membrane of claim 35 which is suitable for electrodialysis, diffusion dialysis and Donnan dialysis applications wherein the quantity of in-situ polymerized polyelectrolyte or hydrogel is about 50 to about 250 wt% and the amount of cross-linking monomers is from about 0.25 to about 30 wt% of the in-situ polymerized monomers.

39. The charged membrane of claim 38 wherein the quantity of in-situ polymerized polyelectrolyte or hydrogel is about 150 to about 250 wt% and the amount of cross-linking monomers is about 15 to about 25 wt%.

Pervaporation with Water/Ethanol Mixtures:
Effect of Temperature

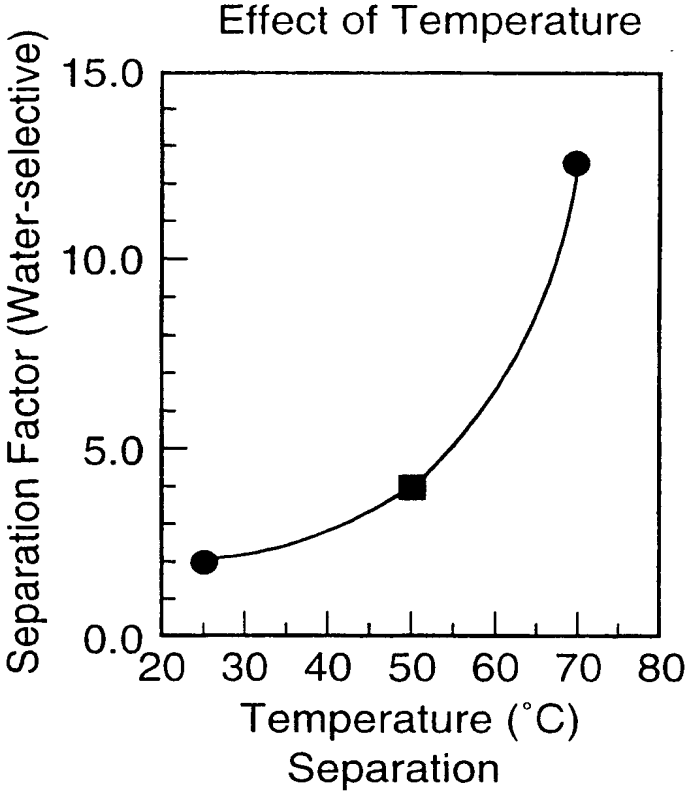

FIG.1A

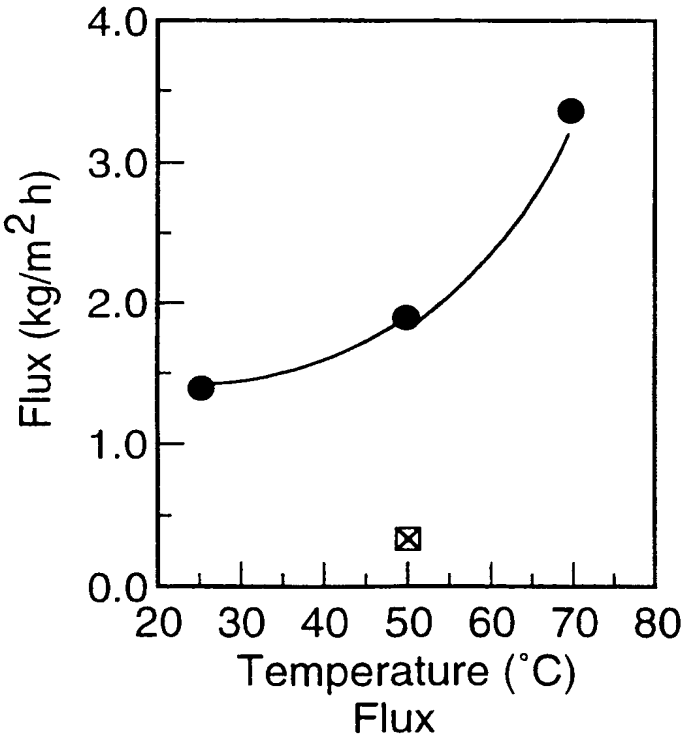

FIG.1B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00770

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01D69/10 B01D69/14 B01D67/00 B01D71/28 B01D71/78
B01D71/80 B01D71/82

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 97 17129 A (UNIV TOLEDO) 15 May 1997 see page 8, line 5 - page 8, line 13 see page 8, line 21 - page 9, line 15; claim 1 --- -/--	1,18-20, 26,35,36

[X] Further documents are listed in the continuation of box C.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
B01D 69/10, 69/14, 67/00, 71/28, 71/78, 71/80, 71/82

A1

(11) International Publication Number: WO 98/17377

(43) International Publication Date: 30 April 1998 (30.04.98)

(21) International Application Number: PCT/CA97/00770

(22) International Filing Date: 17 October 1997 (17.10.97)

(30) Priority Data:
08/733,792 18 October 1996 (18.10.96) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 08/733,792 (CIP)
Filed on 18 October 1996 (18.10.96)

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MICROPOROUS MEMBRANES AND USES THEREOF

(57) Abstract

Charged membranes comprise a porous substrate and a cross–linked polyelectrolyte or hydrogel located in the pores of the substrate and are useful in a variety of membrane separation processes, including pressure driven membrane separation, diffusion dialysis, Donnan dialysis, electrodialysis, electrochemical synthesis and pervaporation.

*(Referred to in PCT Gazette No. 38/1998, Section II)

BNSDOCID: <WO___9817377A1_IA>

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